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**EFFECT OF SUPPLEMENTAL CALCIUM AND VITAMIN D IN
FEEDLOT DIETS ON BEEF TENDERNESS**

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Effect of Supplemental Calcium and Vitamin D in Feedlot Diets on Beef Tenderness

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Final Report

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Executive Summary:

This report details the final results of the project "Effect of Supplemental Calcium and Vitamin D in Feedlot Diets on Beef Tenderness". The rationale for this study is based on American research that has shown that feeding mega levels of vitamin D3 (> 5 million IU/head/day) to beef cattle prior to slaughter improves beef tenderness. The theory is that high levels of vitamin D3 in the diet lead to elevated plasma calcium levels at slaughter which in turn activate calcium dependant proteases in the carcass following slaughter. These proteases are important in post-slaughter "aging" of the carcass and ultimately in tenderness of the beef. Feeding high levels of vitamin D3 however, can lead to carcass residues which could harm consumers and potential side effects in cattle. It would be desirable to achieve this improvement in tenderness without having to feed such high levels of this vitamin to cattle. The objectives of this work were to:

1. study how plasma calcium levels can be manipulated by dietary means such that we can reduce the level of vitamin D3 in the diet required to improve beef tenderness. This is particularly important in light of possible tissue residue concerns.
2. explore the metabolic response to high levels of vitamin D3 in an effort to more fully understand how plasma calcium levels are influenced by vitamin D3 feeding.
3. determine the effectiveness of vitamin D3 supplementation under Western Canada (environment, feedstuffs, management, etc.) conditions.

To achieve these objectives, a series of 4 experiments were carried out. These included:

Experiment 1: Effect of supplemental vitamin D3 on plasma calcium levels in finishing beef steers

Experiment 2: Supplementing high levels of vitamin D₃ on the homeostatic control of serum calcium in finishing steers.

Experiment 3: Effect of feeding a low calcium diet and vitamin D₃ on calcium metabolism in finishing steers.

Experiment 4: Effect of low and high calcium diets, anionic salts and vitamin D₃ supplementation to finishing beef steers on calcium and acid-base homeostasis.

The results of this research are of interest to the Canadian cattle industry, whose focus continues to be production of a safe, wholesome product that meets consumer demands. The results of experiments 1 and 2 show that when high levels of vitamin D₃ are fed under conditions common to western Canada, the plasma calcium response is directly proportional to the amount of vitamin D₃ fed. When fed in combinations with barley grain and barley silage, it was necessary to provide 5 million IU of vitamin D₃ per head for 7 days prior to slaughter to achieve plasma calcium levels comparable to that seen in the US which triggered an improvement in tenderness of the beef. Metabolic profiles of plasma however, showed extremely high circulating levels of vitamin D₃ and its metabolites, particularly 25(OH)D₃. Since these forms of vitamin D are stored in fat and liver tissue, these high levels would pose residue problems for consumers. In light of the documented potential for residues of vitamin D₃ in organ and tissues, it is doubtful if feeding this level of vitamin D₃ to cattle to improve beef tenderness would ever be approved by Canadian regulatory agencies. It is therefore of interest to explore alternative mechanisms to increase serum Ca levels that may work either alone or in combination with vitamin D₃ to improve beef tenderness.

Experiments 3 and 4 utilized manipulation of dietary calcium levels and the feeding of anionic salts in an attempt to "trick" the metabolism of the animal. The intent was to see if it was possible through dietary manipulation to reduce the level of vitamin D₃ needed to obtain a rise in plasma calcium that is high enough to trigger improved beef tenderness. Such a method would lead to lower carcass residues of vitamin D₃ and its metabolites. Experiment 3 used the approach commonly used in dairy herds to combat milk fever, that is feed a low calcium diet for a set period of time in order to activate calcium absorption mechanisms. Although there was evidence that feeding a low calcium diet primed the calcium absorption mechanisms of the animal, there was no synergistic interaction between the feeding of a low calcium diet and vitamin D₃ feeding. Feeding the low calcium

diet in combination with reduced levels of vitamin D3, did not raise plasma calcium levels to those obtained with feeding 5 million IU of vitamin D3 per head per day. Experiment 4 used a combination of feeding a low calcium diet with anionic salt supplementation and then the feeding of grade levels of vitamin D3. This approach was also designed to stimulate calcium absorption mechanisms and enhance the effect of vitamin D3 feeding. The results of this study showed that feeding the anionic salts in combination with either 1.25 or 2.5 million IU of vitamin D3 resulted in a rise in plasma calcium levels equal to that observed with the feeding of 5 million units of vitamin D3 per head per day. In effect the level of vitamin D3 was reduced by half, that was required to raise plasma calcium to levels seen where beef tenderness is improved. These results hold potential for improvement of beef tenderness through a combination of vitamin D3 feeding and anionic salt supplementation. However, further research is required to determine the potential for any residues of vitamin D3 that may result from this practice.

The project formed the basis of a Ph.D. program for Mr. Gilberto Aranda-Osorio. Due to the nature of this work it was necessary to apply to the Bureau of Veterinary Drugs for an experimental studies certificate. This was undertaken. Development of high performance liquid chromatography methodology for measurement of vitamin D and 25-hydroxy and 1-25 dihydroxy vitamin D was undertaken over the summer/fall of 1999 and winter of 1999/2000. This work was carried out by Dr. Olkowski (Professional Research Associate) and Mr. Aranda - Osorio.

1. Introduction:

Tenderness is an important component of meat quality. Inconsistency in meat tenderness has been identified as one of the major problems facing the beef industry. It has been estimated that the economic loss associated with beef toughness equals \$7.64 per animal or \$217 million US annually to the beef industry (Smith et al. 1995; cited by Swanek et al. 1997).

Differences in the rate and extent of postmortem tenderization is in part responsible for the variation in meat tenderness at the consumer level. Breakdown of key muscle proteins by calcium dependant neutral proteases (calpains) is responsible for meat tenderization that occurs during postmortem storage of meat (Koochmaraie, 1990).

It was found that injecting meat with calcium chloride increases tenderness through activation of the calpain proteolytic system. Exogenous calcium applied to prerigor muscles activates both μ -calpain and m-calpain, accelerates aging, and enhances meat tenderness (Wheeler et al. 1992).

These enzymes, μ -calpain and m-calpain, are dependent on calcium concentration for activity. Their post-rigor activity may be enhanced by increasing plasma calcium concentration, thus leading to increased proteolysis and improved meat tenderness (Owens et al. 1998).

In the live animal, calcium is tightly regulated by calciotropic hormones (vitamin D3, parathyroid hormone, and Calcitonin). Among them, vitamin D3 (hormonal form, 1,25(OH)₂D₃), plays a important role in elevating extracellular fluid calcium levels if they are low by increasing calcium absorption from the gut, resorption from bone and reabsorption from kidney (Mundy, 1990).

Supplemental levels of vitamin D3 at levels in excess of those currently fed to beef cattle have been given to cattle in order to increase plasma calcium levels and thus improve beef tenderness. Swanek et al. (1997) evaluated the effects of supplemental vitamin D3 on meat tenderness. Beef steers were supplemented with 0, 5 or 7.5 million IU vitamin D3 per head per day for either 5 or 10 days immediately prior to slaughter. Supplementation resulted in a reduction in shear force values and a reduction in the number of tough steaks after 7 and 14 days of postmortem aging. Plasma calcium concentration at slaughter was greater for steers that received supplemental vitamin D3

(control, 9.23 mg/ml; 5 million IU, 10.39 mg/ml; value for 7.5 million IU was not reported). These authors concluded that increased activity of proteases associated with the calpain system during aging was responsible for increased meat tenderness.

Montgomery et al. (1998) conducted an experiment in which 5 or 7.5 million IU vitamin D3 was administered to finishing steers daily, for 9 days. Cattle were slaughtered 2 days later. Blood samples were collected daily and at slaughter for plasma calcium quantification. Warner-Bratzler shear force and sensory evaluation were determined. The two groups fed supplemental vitamin D3 had higher concentrations of plasma calcium from d 5 to d 8 (control, 8.8; 5 million IU, 11; and 7.5 million IU, 12.2 mg/dl). Steaks (aged 14 d) from cattle administered 5 million IU vitamin D3 had higher concentrations of total muscle calcium (0.34 mg/g) than that did steaks from controls (0.25 mg/g) or cattle treated with 7.5 million IU of vitamin D3 (0.28 mg/g). These authors concluded that the 5 million IU vitamin D3 per day was equally effective to 7.5 million IU per day in improving beef tenderness.

Two important areas that have not been researched include:

a. *What is the level of Hypercalcemic Agents that is effective?*

American studies have shown that 5 to 7 million IU of vitamin D3 for 5 to 10 days has been effective in improving beef tenderness. There is no work reported that has shown the effect of feeding lower levels of vitamin D3 on beef tenderness. There is also no work studying the interaction of dietary manipulation of calcium intake (as in the case of milk fever with dairy cows) with various levels of supplemental vitamin D3 feeding. Similarly the feeding of anionic salts to dairy cattle has been shown to reduce the incidence of milk fever. No research has been conducted however, with the feeding of anionic salts with lower levels of vitamin D3 to see if plasma calcium levels can be evaluated to levels that are compatible with those associated with improved beef tenderness.

b. *Potential for tissue residues of vitamin D3 and its metabolites*

There are no published studies of tissue residues of vitamin D3 and its main metabolites following supplementation of beef steers with this vitamin at levels targeted to improved beef tenderness. Unpublished work from, Montgomery et al. and Owens et al. found that supplementing 7.5 million IU vitamin D3 per day for 5 to 7 days to finishing

steers , increased liver vitamin D3 levels to 3500 to 4000 IU/ 100 grams relative to 30 to 40 IU/ 100 grams in livers from control animals. Kidney levels increased from approximately 30 IU per 100 grams in control animals to 800 to 1100 IU / 100 grams. Increases in fat and muscle tissue while noted were much smaller in magnitude (i.e. muscle tissue – 16 IU / 100 gram in control to 364 IU / 100 grams in treated animals). Canadian research is required to determine residue levels in tissues of cattle fed high levels of vitamin D. Specifically, is it possible through dietary manipulation to reduce the level of vitamin D3 fed and thus reduce residue problems.

2. Objectives of Research:

1. It is clear that supplementing high levels of vitamin D3 effectively increase plasma calcium concentrations and leads to improved beef quality. One of the primary objectives of our work was to study how plasma calcium levels can be manipulated by dietary means such that we can reduce the level of vitamin D3 in the diet required to improve beef tenderness. This is particularly important in light of possible tissue residue concerns. We are interested in the following questions:
 - a. Can plasma calcium levels be increased by levels of vitamin D3 supplementation that are less than the 5 to 7 million IU units per head per day that has been shown to be effective in the American research. Can this be accomplished by manipulating dietary calcium or anionic salt intake (as is common in dairy herds with milk fever problems) in combination with vitamin D3 supplementation.
 - b. Can tissue residues be reduced by feeding lower levels of vitamin D3 in combination with manipulation of dietary calcium feeding.
2. There is little evidence at present that explains how high levels of vitamin D3 supplementation influence plasma levels of calcium and vitamin D3 and its metabolites. This work will explore the metabolic response to high levels of vitamin

D3 in an effort to more fully understand how plasma calcium levels are influenced by vitamin D3 feeding.

3. We are interested in determining the effectiveness of vitamin D3 supplementation under Western Canada (environment, feedstuffs, management, etc.) conditions.

3. Research Work:

Experiment 1: Effect of supplemental vitamin D3 on plasma calcium levels in finishing beef steers

Objectives:

The objective of this study was to investigate the effects of feeding graded levels (1.25, 2.5 and 5 MIU/hd/d for seven days) of vitamin D₃ on serum total and ionized Ca levels and on feed intake of cattle fed diets common to Western Canada.

Material and Methods:

Experimental Design:

Adaptation Period (A1 to A33):

Fifteen Angus steers (452 ± 28 kg) were housed in individual pens (3.6 x 3.6 m) with automatic water bowls in the Livestock Research Barn of the Department of Animal and Poultry Science at the University of Saskatchewan. Animals were cared according to the guidelines of the Canadian Council of Animal Care. The cattle were adapted to the experimental diet for 33 days. The ration consisted of 75% concentrate (barley grain base) and 25% barley silage (as fed) meeting the requirements for this type of cattle according to the National Research Council (NRC 1996)(Table 3.1). Feed was offered twice a day (0700 and 1600 h), with orts collected and weighed everyday before the morning feeding. Individual maximum voluntary intake was measured from day A20 to A26. Then, voluntary intake was then restricted to 85% from days A27 to A33 to allow for cattle to even their intake and consume all daily feed offered. Initial blood samples were obtained by jugular venipuncture on day A17, A24 and A31 at approximately 1300 h. These samples served as the pre-treatment control samples. Blood samples were collected into 7ml-draw evacuated (Vacutainer®) tubes without anticoagulant, blood was then allowed to clot at room temperature for approximately 2 h, then they were centrifuged at 3500 rpm for 15 min. The serum was then used for the determination of total and ionized Ca concentrations.

Supplemental Period (S1 to S7):

During this period, the diet and the feeding pattern were the same as in the last week of the adaptation period (feed restriction to 85%, two feedings a day). Feed intake was monitored daily, with orts collected and weighed everyday before the morning feeding. The steers were randomly assigned to one of the following vitamin D₃ supplemental treatments: 1.25, 2.5, and 5 MIU of vitamin D₃ per steer per day, for seven consecutive days, with five steers per treatment. The vitamin D₃ supplement was prepared from the vitamin D₃ premix containing 500,000 IU per gram (Rhone-Poulenc Canada Inc.), using ground barley as a carrier. The vitamin D₃ supplement was formulated to have a final concentration of 13.0 MIU D₃ per kg of supplement. This supplement was analyzed (New Jersey Feed Laboratory, Inc.) and the actual concentration was 13.13 ± 0.15 MIU D₃ per kg. The total daily amount of vitamin D₃ supplement assigned per steer per day was equally divided and supplied at each feeding. The supplement was top-dressed onto the ration and thoroughly mixed by hand before delivering into the feedbunk. Daily blood samples (from day S1 to S7) were obtained and processed from each steer, under the same protocol as in the previous period.

Withdrawal Period (W1 to W8):

Once vitamin D₃ supplementation ended, the steers were maintained for five days under the same experimental conditions to evaluate post-treatment effects. Immediately after the bleeding on day W5, the cattle were shipped back to the feedlot. During this period, feed was offered without restriction according to their individual daily consumption. Similarly, feed intake was daily monitored with orts collected and weighed before every morning feeding. Blood samples were obtained from day W1 to W5 in the metabolism barn, and on day W8 at the feedlot. Blood sampling was carried out under the same protocol as in previous periods.

Laboratory Analyses: Total Calcium Determination:

Total Ca in serum was determined by indirect potentiometry utilizing a Ca ion selective electrode in conjunction with a sodium reference electrode under the SYNCHRON LX System (Beckman Coulter, Inc., Fullerton, CA. USA). Total Ca was

calculated from free Ca once the molar ratio between free and total Ca concentrations was constant. To achieve a constant molar ratio between total and free Ca, a buffered solution (which contains strong Ca complexing agents) was used. The ratio was one part serum to 21 parts buffered solution (LX ISE Electrolyte Buffer Reagent: Tris). The high molar strength buffer served to establish a constant activity coefficient for Ca ions. The electrode was then calibrated to standard concentration values, and unknowns calculated.

Ionized Calcium Determination:

Ionized Ca in serum was determined by the use of an ion selective electrode (Radiometer ABL 700 Series Analyzer, Copenhagen). The analyzer measures the differential in voltage between a reference electrode and a Ca ion-selective electrode in a solution. The potential established by the unknown solution is compared with the potential developed by a standard solution of known ionic strength and ion composition. Three electrodes are seated in a temperature-controlled module (37°C). The serum sample moves along the sample path by peristaltic pumps. The pH of the serum was measured by a pH glass and reference electrode, adjusting ionized Ca values to pH 7.4 (Gases Ionized Calcium, Operator's Manual, Ciba-Corning 634 Ionized Ca/pH system).

Statistical Analysis:

The data was analyzed using the Analysis of Variance Technique including Repeated Measures Analysis using the General Linear Model procedure of SAS (SAS 1989) to compare treatments within days and over time (across the experimental periods). Contrast of interest included:

- 1) Pre-treatment control value vs the 1.25 MIU treatment.
- 2) Pre-treatment control value vs the 2.5 MIU treatment.
- 3) Pre-treatment control value vs the 5.0 MIU treatment.

Results and Discussion:

Feed Intake (DMI):

Voluntary individual intake calculated from day A18 to A24 of the adaptation period averaged 10.83 ± 1.42 kg of dry matter (DM). Feed intake was then restricted to 85% of *ad libitum* for days A25 to A31. The aim was to eliminate as much as possible any daily fluctuation. Variation in intake during the supplemental period could then be considered a direct effect of treatment.

Vitamin D₃ supplementation depressed ($P < 0.05$) feed consumption from day 6 of supplementation (S6) to approximately day 3 of the withdrawal period (W3) for all the treatments (Fig. 1.1). Minimum intake values were attained on day W1, and they represented an 18.7, 27.7, and 36.7 % reduction ($P < 0.05$) in feed consumption with respect to the average pre-treatment value for the 1.25, 2.5 and 5.0 MIU treatments, respectively. No differences ($P > 0.05$) among treatments were detected. Feed intake tended to recover after supplementation, however, intakes were still lower than pre-treatment values, 4 days after supplementation (Fig. 1.1). The depression in feed intake observed in the present study influenced the total amount of vitamin D₃ consumed. Total vitamin D₃ consumption averaged 96.1 % (1.20 MIU/d), 90.3 % (2.26 MIU/d) and 89.4 % (4.48 MIU/d) for the 1.25, 2.5 and 5 MIU treatments, respectively.

Total and Ionized Serum Ca:

Serum levels of the three pre-treatment control samples for total and ionized Ca concentrations were 10.61 ± 0.35 and 5.12 ± 0.15 mg/dl, respectively (Fig. 1.2 & 1.3). Normal Ca concentration in blood ranges between 9.0 to 11.0 mg/dl and from 4.8 to 6.4 mg/dl, for total and ionized Ca, respectively. Thus, the total and ionized serum Ca concentrations found in the present experiment appeared to be in good agreement with the normal ranges reported in the literature.

In this study, it was found that supplementation of vitamin D₃ did not influence ($P > 0.05$) total Ca concentrations relative to the control period during the first three days of supplementation, although a slight ($P < 0.05$) effect of treatment was evident. For the 2.5 and 5 MIU treatments, total Ca concentration was increased ($P < 0.0001$) from day 4 of supplementation (S4) until day 5 of the withdrawal (W5) period. Even the concentration on

day W8, while decreased was still higher ($P < 0.05$) than pre-treatment control values. In contrast, total Ca concentrations for the 1.25 MIU treatment were not different ($P > 0.05$) from pre-treatment values throughout the experiment (Fig. 1.2).

Parallel changes were observed in regard to ionized Ca. However, increments were significant ($P < 0.05$) from day S4 to W5, for the 2.5 and 5 MIU treatments. On day W8, ionized Ca concentrations returned to normal levels for all treatments. The 1.25 MIU treatment had similar ($P > 0.05$) serum ionized Ca levels as the pre-treatment control value throughout the experiment (Fig. 1.3).

Maximum total Ca concentrations were 11.03, 11.67 and 12.43 mg/dl, which represented an increase in 4.0, 10.0 and 17.2 % with respect to the adaptation period, for the 1.25 (on day W4), 2.5 (on day W3) and 5.0 (on day W5) MIU treatments, respectively (Fig. 1.2). Maximum ionized Ca concentrations were 5.39, 5.73 and 6.03 mg/dl, which represented an increase of 5.3, 11.9 and 17.8 % with respect to the pre-treatment control value for the 1.25 (on day W4), 2.5 (on day W2) and 5.0 (on day W4) MIU treatments, respectively (Fig. 1.3).

Comparing these results with the literature, it was found that the total Ca concentration found with the 5 MIU treatment was higher than the 10.39 mg/dl reported by Swanek *et al.* (1997) who also supplemented 5 MIU D_3 to finishing steers for 5 days. This difference can be explained firstly, by the number of days of supplementation (7 vs 5) and, secondly, because the steers in their study were sacrificed immediately after supplementation without a withdrawal period. In the present study, maximum values were attained during the withdrawal period. In a subsequent study, Swanek *et al.* (1999) reported a plasma Ca value of 12.39 mg/dl, when 5 MIU D_3 was supplemented for 7 days. Results that are in complete agreement with this study. Increments of approximately 2 mg/dl in total Ca concentration as a result of 5 MIU D_3 supplementation have also been reported by Montgomery *et al.* (1998 and 2000) and Karges *et al.* (1999a,c).

The rise in total and ionized Ca levels observed in this study has important implications for the beef industry. Other workers have observed that high levels of vitamin D_3 supplementation induced higher plasma Ca concentrations, which in turn produced higher muscle Ca concentrations (Montgomery *et al.* 1998, 2000 and in press; Karges and Morgan 1999; Swanek *et al.* 1999). This excess of Ca in muscle is thought to stimulate

higher activity of the calpain proteolytic enzyme system (m-calpain, μ -calpain), which leads to more tender beef (Koochmaraie 1992, 1996; Zamora *et al.* 1998). Swanek *et al.* (1997) who supplemented 5 MIU D₃ for 5 days and observed a rise in plasma Ca of 12.6 %. This increase in Ca was enough to reduce shear force values by 6.6% and the number of tough steaks by 21.8 % (seven days postmortem aging). Similar improvements in shear force values and tenderness in response to vitamin D₃ supplementation, which elicited similar increases in plasma Ca as observed in this study, were observed by Swanek *et al.* (1999) and Montgomery *et al.* (2000).

The results of this study showed that the rise in serum Ca is related to the level of vitamin D₃ supplementation. Minimal increases were seen with the 1.25 MIU treatment, in contrast the greatest increases were seen with the 5 MIU treatment. To more fully understand the response of serum Ca to vitamin D₃ supplementation and to possibly manipulate the response, it is necessary to more fully understand the biological mechanism that leads to this rise including the role of the calcitrophic hormones such parathyroid hormone (PTH), calcitonin and vitamin D₃ and its major metabolites.

Conclusions:

The results of this study showed that feed intake was depressed as a result of vitamin D₃ supplementation, and the depression followed an inverse relationship with the amount of vitamin D₃ supplemented. Intake recovery was observed only after supplementation ended, with the rate of recovery being related to the degree of depression. From these observations and from other studies, it was concluded that the depression in feed intake when high levels of vitamin D₃ are supplemented is due to metabolic or physiological alterations and not to palatability factors.

The levels of supplemental vitamin D₃ used in this study have been shown to be an effective way to increase serum Ca concentrations in beef steers fed with a common barley grain-based feedlot finishing diet. The observed increments in serum Ca were in direct relationship with the amount of vitamin D₃ supplemented, that is, higher levels produced larger increments.

Interestingly, the maximum increments in total and ionized serum Ca concentrations from supplemented steers were found not during the supplemental but during the withdrawal period. This post-treatment rise may result from alterations in the hormonal control of Ca metabolism, a phenomenon that deserves further investigation.

The results of this study where barley grain-based finishing rations were fed have important implications for the beef industry in Western Canada. The rise in serum Ca obtained in this study with the 5 MIU treatment is similar to that noted by other workers who with corn-based diets have noted a considerable improvement in beef quality, notably a reduction in shear force values. If this can be achieved for Canadian beef, particularly at lower levels of vitamin D₃ supplementation, then it is possible that such dietary manipulation of Ca homeostasis may become a common management tool.

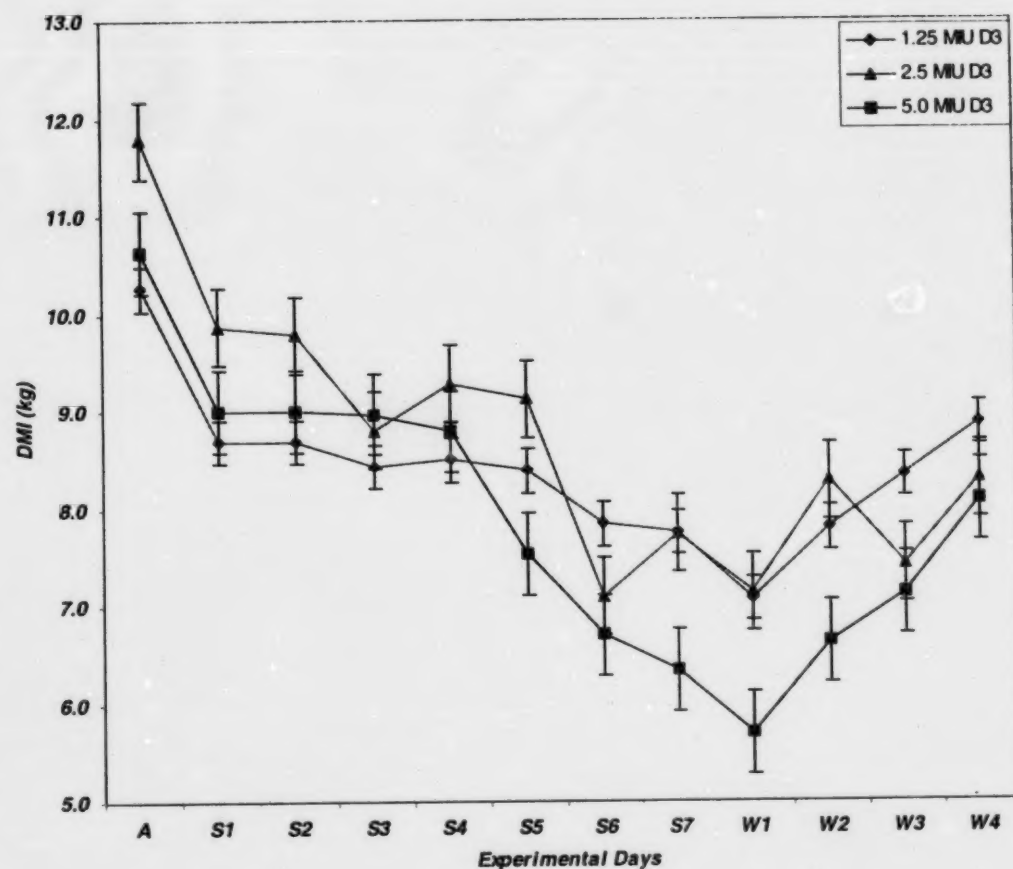


Figure 1.1. Effect of supplementing high levels of vitamin D₃ on feed intake (DMI) of finishing Angus steers (452 ± 30 kg BW), during pre-treatment (A), treatment (S1 to S7) and post-treatment (W1 to W4) periods.

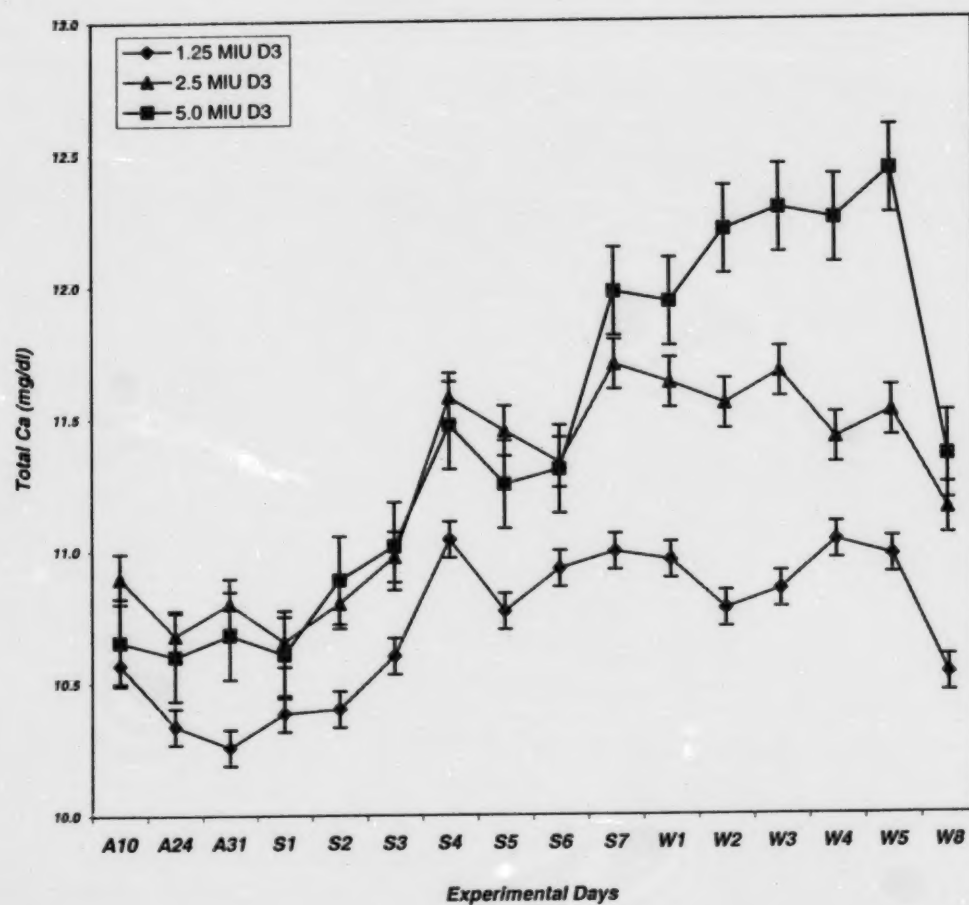


Figure 1.2. Effect of supplementing high levels of vitamin D₃ on total serum Ca concentrations of finishing Angus steers (452 ± 30 kg BW), during pre-treatment (A1 to A31), treatment (S1 to S7), and post-treatment (W1 to W8) periods.

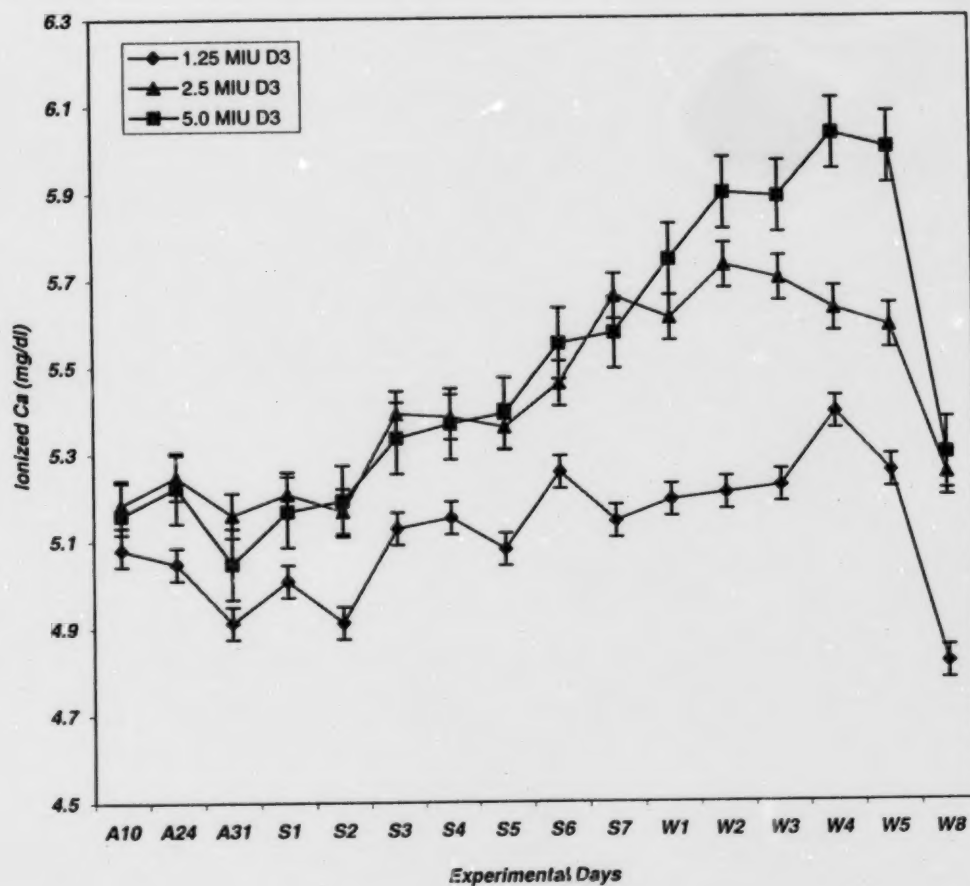


Figure 1.3. Effect of supplementing high levels of vitamin D₃ on ionized serum Ca concentrations of finishing Angus steers (452 ± 30 kg BW), during pre-treatment (A1 to A31), treatment (S1 to S7), and post-treatment (W1 to W8) periods.

Experiment 2. Supplementing High Levels of Vitamin D₃ on the Homeostatic Control of Serum Calcium in Finishing Steers.

Objectives:

The objectives of this study were to evaluate the effect of supplementing high levels of vitamin D₃ (0, 2.5 and 5 MIU per day for seven consecutive days) on the following parameters (during supplemental and post-treatment periods):

- 1) Total and ionized serum Ca concentrations, and the ionized to total Ca ratio;
- 2) Vitamin D₃ and 25-hydroxyvitamin D₃ plasma concentrations;
- 3) Parathyroid hormone and calcitonin serum concentrations;
- 4) Feed intake.

Material and Methods:

Experimental Design:

Adaptation Period (A1 to A18):

Fifteen Hereford steers (607 ± 12 kg BW) were housed in individual pens (3.6 x 3.6 m) with automatic water bowls in the Livestock Research Barn of the Department of Animal and Poultry Science at the University of Saskatchewan. Animals were cared according to the guidelines of the Canadian Council of Animal Care. The cattle were adapted to the experimental diet for 18 days. The diet consisted of 75% concentrate (barley grain base) and 25% barley silage (as fed), meeting the requirements for this type of cattle according to the National Research Council (1996)(Table 4.1). The steers were fed twice a day (0800 and 1600 h), withorts collected and weighed every day before the morning feeding. Individual maximum voluntary feed intake was measured from days A5 to A11. Subsequently, feed was offered at this level from days A12 to A18, with the objective to level out intake before vitamin D₃ supplementation and so be able to measure its effect on feed intake.

Supplemental Period (S1 to S7):

The steers were randomly assigned to one of the following supplemental treatments: 0, 2.5, or 5.0 million IU (MIU) of vitamin D₃ per steer per day for seven consecutive days, with five steers per treatment. The vitamin D₃ supplement was prepared from a vitamin D₃ concentrate containing 500,000 IU per gram (Rhone-Poulec Canada Inc.), using ground barley as a carrier. This supplement was formulated to have a final concentration of 13.0 MIU D₃ per kg of premix. The total daily amount of supplement per steer per treatment was equally divided and supplied at each feeding. The supplement was top-dressed to the ration and thoroughly mixed by hand before delivering it into the feed bunk. During this period, daily blood samples (S1 to S7) were obtained by jugular vein, from all the steers, at approximately 1300 h. Three sets of samples were taken. For the first and second sets, blood was collected into 7ml-draw evacuated (Vacutainer®) tubes without anticoagulant, the blood obtained was allowed to clot at room temperature for approximately 2 h. Then, one set was centrifuged at 3500 rpm for 15 min, and the serum obtained was immediately used to determine total and ionized Ca concentrations. The second set was centrifuged at 3000 rpm for 10 min at -4°C, then the serum was collected and transferred to 1.7 ml centrifuge clear vials and stored at -70°C until analysis for PTH and calcitonin concentrations. For the third set, blood was collected into 7 ml-draw vacutainer tubes with anticoagulant (100 USP units of lithium heparin), which were placed in crushed ice immediately after withdrawal, and approximately 2 h later centrifuged at 3000 rpm for 10 min at -4°C, then the plasma was collected and transferred to 1.7 ml centrifuge clear vials and stored at -70°C until analysis for vitamin D₃ and 25(OH)D₃ concentrations.

Withdrawal Period (W1 to W9):

Once the vitamin D₃ supplementation period ended, the steers were maintained for a further five days under the experimental conditions to evaluate post-treatment effects, then the steers were shipped back to the feedlot. Feed was offered without restriction and was monitored daily with orts collected and weighed before every morning feeding. Daily blood samples were obtained from day W1 to W5, and a final sample on day W9, under the same protocol as described previously.

Laboratory Analyses:**Total and Ionized Serum Calcium Determinations:**

Serum samples were analyzed for total and ionized Ca as described in experiment

2.

Parathyroid Hormone (PTH) Determination:

Serum concentrations of PTH were analyzed by a two-site immunoradiometric assay (IRMA)(Intact PTH- Parathyroid Hormone. Nichols Institute Diagnostics). Two different goat polyclonal antibodies to human PTH, purified to be specific to two different regions of the PTH molecule were used. One antibody bound the mid-region and C-terminal PTH 38-84, this antibody was immobilized onto plastic beads. The other antibody bound the N-terminal PTH 1-34, this was radiolabeled for detection.

Calcitonin Hormone Determination:

Calcitonin (thyrocalcitonin) concentrations in serum were determined by a double – antibody radioimmunoassay (Double Antibody Calcitonin. Diagnostic Products Corporation DPC®, Los Angeles, CA. USA). This is a sequential competitive radioimmunoassay procedure. The samples were first pre-incubated with anti-calcitonin antiserum. ¹²⁵I-labeled calcitonin then competed with calcitonin in the sample for antibody sites. After incubation for a fixed period of time (18 h), separation of bound from free was achieved by the PEG-accelerated double-antibody method. Finally, the antibody-bound fraction was precipitated and counted by a gamma counter. Calcitonin concentration in the samples was read from a calibration curve.

Vitamin D₃ and 25-Hydroxyvitamin D₃ (25-OH) Determinations:

Plasma concentrations of vitamin D₃ and 25(OH) were carried out under reverse-phase high-performance liquid chromatography (HPLC) assay. These two compounds were extracted from plasma samples by using acetonitrile (1:2 ratio). Solutions were thoroughly mixed (vortexed) for a fix period of time, then centrifuge. The supernatant from

control animals undergo a second extraction with chloroform and hexane and evaporation under nitrogen and then reconstitution with acetonitrile. The supernatant from treated steers undergo only the first extraction. Supernatants were filtered and transferred to injection vials. Samples were injected onto the HPLC (Waters™, Millipore Co. Milford, MA, USA) using a reverse-phase column (Phenomenex C18 3μ 15 x 4.6 mm) and acetonitrile as the mobile phase under isocratic conditions. The metabolite 25(OH)D₃ was eluted first and vitamin D₃ minutes later. The absorbance of these analytes (peak area) was computed and integrated by the software Millenium 2010 (Millenium™, Millipore Co. Milford, MA, USA). Calibration curves were used to calculate concentrations from the experimental samples.

Statistical Analysis:

The feed intake and blood sample data were analyzed using the Analysis of Variance Technique including Repeated Measures Analysis using the General Linear Model procedure of SAS (SAS 1989) to compare treatments within days and over time. Contrast of interest included:

- 1) Control versus the average response of the two vitamin D₃ treatments.
- 2) Comparison between the 2.5 and 5 MIU D₃ treatments.

Results and Discussion:

Feed Intake (DMI):

Feed intake during the adaptation period averaged 8.99 ± 1.21 kg of DM. Intake from control animals was maintained without change throughout the supplemental and withdrawal periods. During the first days of the supplementation period, feed intake was not different ($P > 0.05$) between control and supplemented steers, with the exception on the second day of supplementation (S2) where it was highly depressed ($P < 0.05$) in the 5 MIU treatment, but it recovered the following day. The trend over time was to reduce ($P < 0.05$) feed intake as supplementation advanced with no difference ($P > 0.05$) between levels of supplementation. Feed intake tended to recover after supplementation ended, following an inverse relationship with the level of vitamin D₃ supplemented, although it had not fully recovered four days post-treatment. Minimum intakes were attained towards the end of

the supplemental period, which represented a decreased of 18.2 and 37.2 % relative to control animals for the 2.5 and 5 MIU treatments, respectively (Table 2.1). Depression of feed intake as a result of high levels of vitamin D₃ supplementation was evidenced in the previous study and this response has also been observed in other studies conducted in the United States.

The vitamin D₃ supplement was formulated to have a final concentration of 13.0 MIU per kg. Analysis of the supplement indicated an actual concentration of 12.90 ± 0.46 MIU D₃ per kg (New Jersey Feed Laboratory Inc. Trenton, New Jersey). As the supplement was added to the daily ration, vitamin D₃ consumption was directly related to daily feed intake. As such, the average vitamin D₃ consumption for the 2.5 and 5 MIU treatments were 85.4 % (2.14 MIU/d) and 68.38 % (3.41 MIU/d) of the targeted intakes, respectively.

Total and Ionized Serum Ca Concentrations:

Total and ionized serum Ca concentrations on the first day of supplementation were 10.11, 10.18 and 10.26 mg/dl and 4.92, 5.08 and 5.06 mg/dl for the 0, 2.5 and 5 MIU treatments, respectively, with no difference ($P>0.05$) between treatments (Figs. 2.1 and 2.2). These initial concentrations, as well as the range from 9.90 to 10.19 mg/dl for total and 4.78 to 5.11 mg/dl for ionized Ca from control animals during the whole experiment were in the normal range for cattle.

Total and ionized serum Ca concentrations were increased ($P<0.0001$) as a result of vitamin D₃ supplementation. Contrast analysis indicated that Ca serum concentrations in supplemented animals were higher ($P<0.05$) than from controls, from day S3 to W5 and from S4 to W9 for total and ionized Ca, respectively. Conversely, differences due to treatments (2.5 vs 5 MIU) were only evident ($P<0.05$) during the withdrawal period (from day W2 to W9), with highest levels achieved in the 5 MIU treatment (Figs. 2.1 and 2.2).

It is important to note that maximum Ca concentrations were achieved four days after the end of the vitamin D₃ supplemental period (W4), and were reached the same day for total and ionized Ca for the 2.5 and 5 MIU treatments. The maximum rise in total and ionized serum Ca in response to vitamin D₃ supplementation was similar (11.67 vs 11.02 and 12.43 vs 12.12 mg/dl for total and 5.73 vs 5.36 and 6.03 vs 5.98 mg/dl for ionized Ca) for the 2.5 and 5 MIU treatments in the previous and present study of this series, respectively. Maximum total Ca concentration of 12.12 mg/dl achieved in the present study with the 5 MIU D₃ treatment for seven consecutive days, was higher than the reported by Swanek *et al.* (1997)(10.39 mg/dl), at the same level of supplementation. The difference in values may be due to a lower period of supplementation (5 days), and the fact that the steers in their study were slaughtered immediately after supplementation. In a subsequent study, Swanek *et al.* (1999) supplemented 5 MIU D₃ for seven days, and found a final plasma Ca concentration of 12.39 mg/dl, which was very close to the value found in the present experiment.

Montgomery *et al.* (1998) administered 5 MIU D₃ to finishing steers for eight days and reported that plasma Ca concentrations were increased from 9 to 11 mg/dl. In absolute terms, their final plasma Ca concentration was lower than that found in the

current experiment, even though vitamin D₃ was supplemented by intraruminal boluses. However, the magnitude of the increase was similar as Ca concentration was increased by approximately 2 mg/dl in both studies (from 9 to 11 and from 10.11 to 12.12 mg/dl, respectively). Karges *et al.* (1999a,b) reported a similar rise in plasma Ca when 5 or 6 MIU D₃ were supplemented to steers for twelve (13 mg/dl) or six days (12.12 mg/dl), respectively. It is important to note that the rise in total and ionized Ca levels in serum in this study were similar to that of the studies where this increase has been associated with improved meat quality (Swanek *et al.* 1997, 1999; Karges *et al.* 1999a,b, 2001; Montgomery *et al.* 1998, 2000).

Serum Parathyroid Hormone (PTH) Concentrations:

Serum PTH concentrations of the experimental steers on day S1 were: 147.07, 149.84 and 199.60 pg/ml for the 0, 2.5 and 5 MIU treatments, respectively. Values that were not statistically different ($P>0.05$). Over the course of the supplemental period, PTH values fell in all (supplemented and unsupplemented) steers (Fig. 2.3).

Values for the control steers reached a plateau on day S4, however, for the treated steers they continued to decrease through the supplemental and withdrawal periods. The lowest PTH values attained were 80.75, 19.35 and 8.31 pg/ml on day W5, W5 and W4 for the 0, 2.5 and 5 MIU treatments, respectively, which represented a decrease of 45.0, 87.0 and 96.0 % with respect to their initial values (day S1) for each group (Fig. 2.3).

Contrast analysis indicated that supplemented animals had lower ($P<0.05$) PTH values than control animals from day S6 to W9. However, no differences ($P>0.05$) between levels of supplementation during supplemental or withdrawal periods were identified, with the exception of day S3 and S4, where the 5 MIU treatment had the lowest levels of PTH (Fig. 2.3).

PTH hormone appears to be the principal hormone involved in the minute-to-minute regulation of Ca concentration in blood. It is produced by the chief cells of the parathyroid glands. The parathyroid glands respond to minor fluctuations in Ca concentration by rapidly altering the rate of hormonal secretion and more slowly by altering the rate of hormonal synthesis (Rosol *et al.* 1995). PTH controls plasma Ca concentration by regulating three mechanisms, firstly, by increasing the reabsorption of Ca in the kidneys,

by stimulating the development and activity of bone cells that resorb bone matrix and releasing Ca into blood; and by stimulating the synthesis of the hormonal form of vitamin D₃, which acts on intestinal tissue to increase the efficiency of Ca absorption (Goff 1989; Guyton and Hall 1996). When Ca plasma reaches levels above normal, PTH secretion is reduced, and in acute hypercalcemia the parathyroid glands can suffer hypoplasia (Guyton and Hall 1997). It would appear, therefore, that the reduced levels of PTH in serum were a metabolic adjustment to reduce the high levels of Ca in serum caused by vitamin D₃ supplementation.

Circulating Calcitonin Concentrations:

Serum calcitonin concentrations of the experimental steers were determined on days S1, S3, S6, W1, W3 and W5 (Table 2.2). On the first day of the supplemental period there was no difference ($P>0.05$) in calcitonin concentrations between control and supplemented steers, however, there was ($P<0.05$) between the 2.5 and 5 MIU treatment (Table 2.2). On the third day of supplementation the situation was inversed, that is, there was a difference ($P<0.05$) between control and supplemented steers, but not between vitamin D₃ treatments. From the end of the supplementation period (S6) and throughout withdrawal period, there were no differences ($P>0.05$) between control and vitamin D₃ treatments.

Calcitonin, which is secreted by the parafollicular cells, or C cells of the thyroid gland has the function to reduce blood Ca concentration (Rosol *et al.* 1995). High levels of Ca for long periods of time could result in prolonged increases in blood calcitonin concentrations and in hyperplasia of the C cells, but with almost no affect on the regulation of Ca (Rosol *et al.* 1995). Additionally, these authors pointed out that the function of calcitonin in inhibiting osteoclastic bone resorption is transitory, because the osteoclasts rapidly become refractory to its effect. For this reason the calcitonin regulatory mechanism is considered weaker than the PTH system because its effect only lasts a few hours to a few days at most (Guyton and Hall 1996). This may explain why serum Ca concentrations from supplemented steers were increased and reached high levels for a prolonged period of time, because calcitonin appears to be not as strong as the PTH and 1,25(OH)₂D₃ system to control plasma Ca levels.

Plasma Vitamin D₃ and 25-Hydroxyvitamin D₃ (25(OH)D₃) Concentrations:

Plasma vitamin D₃ concentrations from the experimental steers on the first day of supplementation (S1) were: 4.92, 6.70 and 11.51 ng/ml for the 0, 2.5 and 5 MIU treatments, respectively. Vitamin D₃ concentrations from control animals ranged from 2.71 to 8.20 ng/ml throughout the experiment, with an average of 4.23 ± 1.47 ng/ml (Fig. 2.4). This average was close to the normal values of 1 to 3 ng/ml reported for dairy cattle (McDermott *et al.* 1985; Horst 1986); or the 3.1 ng/ml for finishing beef cattle (Montgomery *et al.* 2000).

Plasma 25(OH)D₃ concentrations from the experimental steers on day S1 were: 50.13, 50.11, 54.03 and 54.03 ng/ml for the 0, 2.5 and 5 MIU treatments, respectively. The 25(OH)D₃ concentrations for the control steers ranged from 43.49 to 60.65 ng/ml throughout the trial, with an average of 48.47 ± 4.33 ng/ml (Fig. 2.5). This average was in good agreement with the normal range of 20 to 60 ng/ml for dairy cattle.

Vitamin D₃ and 25(OH)D₃ were increased ($P < 0.0001$) as a result of vitamin D₃ supplementation, and this response was directly related ($P < 0.0001$) to the level of supplementation, but their responses were different (Figs. 2.4 and 2.5). The increase in vitamin D₃ in plasma fit a normal bell shaped curve. Values increased almost immediately once supplementation started, with a maximum located between days S5 and S7, and declining and plateauing on day W3. At this point, vitamin D₃ concentration remained elevated relative to control values through the end of the trial (W9) (Fig. 2.4). The increase in 25(OH)D₃ concentration was linear from almost the beginning through the end of the trial with the highest value on day W9 (Fig. 2.5). In contrast, the vitamin D₃ and 25(OH)D₃ levels of control animals remained constant throughout the trial.

Maximum vitamin D₃ concentrations were 132.4 and 149.8 ng/ml, which represented a 32.6- and 36.9-fold increase relative to the control animals for the 2.5 and 5 MIU treatments, respectively (Fig. 2.4). Maximum 25(OH)D₃ concentrations were 242.8 and 335.9 ng/ml, which represented a 4.9- and 6.9-fold increase relative to the control animals for the 2.5 and 5 MIU treatments, respectively (Fig. 2.5).

When one considers the metabolic response to supplemental vitamin D₃ it is clear that supplementation caused significant increases in plasma vitamin D₃ and 25(OH)D₃, and

in total and ionized serum Ca, a reduction in PTH and no effect on calcitonin levels. In addition feed intake was dramatically depressed. This rise in total and ionized serum Ca levels is likely a result of several metabolic signals that ultimately influence Ca absorption and resorption. It is clear, first, that PTH levels declined rapidly due to a rise in serum Ca with no detectable affect calcitonin levels. As discussed, this drop in PTH is fully consisted with its metabolic function. Mega levels of dietary vitamin D₃ were found to dramatically increase plasma vitamin D₃ levels. It is obvious that as a result of increased of 25(OH)D₃ levels, the first hydroxylation, which is carried out in the liver by the 25-hydroxylase enzyme was carried out relatively unregulated. Again this is consistent with known functions of this enzyme. The rise in 25(OH)D₃ levels could increase serum Ca levels in one or two fashions. First, increased levels of 25(OH)D₃ could stimulate conversion in the kidney to 1,25(OH)₂D₃ (hormonal form of vitamin D₃), which acts to stimulate Ca binding protein synthesis in intestinal epithelial cells which then increase active Ca transport across the epithelium. In humans and many other species, this step is normally a tightly regulated step with 1,25(OH)₂D₃ levels held in close check (DeLuca 1979; Guyton and Hall 1997). Supporting this reasoning, Montgomery *et al.* (2000), did not find significant changes in 1,25(OH)₂D₃ plasma concentrations in steers supplemented with 5 MIU D₃ for nine days. The second possible mechanism for increased serum Ca levels due to vitamin D₃ supplementation is that 25(OH)D₃ may override the normal process, by performing the functions of 1,25(OH)₂D₃ in intestine and bone by virtue of excess of substrate, that is, although the affinity of the receptors in the target tissues is much higher for 1,25(OH)₂D₃ than for 25(OH)D₃, the excess of 25(OH)D₃, as a result of vitamin D₃ supplementation, could bind the receptors and stimulate the functions of 1,25(OH)₂D₃ in kidney, bone and intestine (Smith *et al.* 1983; Bhagavan 1992).

Finally, it has also been shown in other studies that supplementation with high levels of vitamin D₃ (2.5 and 5 MIU) to finishing steers results in significant increases in plasma vitamin D₃ and 25(OH)D₃ concentrations (Montgomery *et al.* 2000). These higher levels of vitamin D₃ in plasma will lead to higher concentrations in muscle and in other organs (liver) and tissues (adipose) as has been demonstrated by Montgomery *et al.* (2000 and in press). High levels of vitamin D₃ in edible tissues represent the main concern with regard to human health (Owens *et al.* 1998). High residual amounts of vitamin D₃ have

been found in meat, from supplemented animals (5 MIU/d), even after the meat has been cooked (Montgomery *et al.* in press). The residual concern is based on the fact that vitamin D₃ regulates Ca metabolism and elevated consumption (via meat) may lead to alteration in the normal metabolism of Ca in humans, causing, principally, calcification of the soft tissues and formation of stones in kidneys (Hunt and Groff 1990; Guyton and Hall 1996, 1997). Acute intoxication from vitamin D₃ includes lost of appetite, nausea, weakness, hypertention, renal failure, cardiovascular dysfunction and death, which is the result of nephrocalcinosis and heart and aortic calcification (DeLuca 1980; Smith *et al.* 1983; Hunt and Groff, 1990).

In light of the documented potential for residues of vitamin D₃ in organ and tissues, it is doubtful if this dietary treatment to improve beef tenderness would even be approved by Canadian regulatory agencies. It is therefore of interest to explore alternative mechanisms to increase serum Ca levels that may work either alone or in combination with vitamin D₃ to improve beef tenderness.

Conclusions:

Vitamin D₃ supplementation at levels used in this experiment (2.5 and 5 MIU per day) caused a significant depression on feed intake, with the degree of depression and the rate of recovery inversely related to the amount of vitamin D₃ supplemented. Both levels of supplemental vitamin D₃ induced significant increases in total and ionized serum Ca concentrations. The increments were directly related to the level of supplementation. Maximum serum Ca concentrations were attained several days after supplementation ended. This suggests that the cascade of events leading to synthesis of Ca binding protein and subsequently increased Ca absorption were activated, however the mechanisms require time to fully develop. Vitamin D₃ supplementation at both levels, induced a significant increase in plasma vitamin D₃ and 25(OH)D₃ concentrations. These two metabolites had different response curves to supplementation. Plasma vitamin D₃ was increased only during supplementation, whereas 25(OH)D₃ was linearly increased throughout the supplemental and withdrawal periods. This suggests that the activity of the 25-hydroxylase enzyme was not tightly regulated, allowing the conversion of vitamin D₃ to 25(OH)D₃.

The increase in plasma vitamin D₃ and 25(OH)D₃ and subsequently in the total and ionized serum Ca caused a depression in the normal PTH values, although calcitonin was not significantly affected. This suggests that the calciotropic hormone responsible for the increased Ca levels was 1,25(OH)₂D₃, either acting by itself or by the 25(OH)D₃ by virtue of excess of substrate.

Table 2.1 Effect of high levels of vitamin D₃ supplementation on dry matter intake (DMI) of finishing Hereford steers (607 ± 12 kg BW).

DMI (kg)						
Days	Vitamin D ₃ Level (MIU/hd/d)			SEM	Contrasts ^z	
	0	2.5	5.0		C vs D	2.5 vs 5.0
Adaptation Period:						
A	9.20	8.66	9.11	0.57		
Supplemental Period:						
S1	8.57	7.46	8.94	0.65	0.6482	0.1348
S2	8.80	8.92	4.42	0.76	0.0405	0.0012
S3	8.36	8.70	7.75	0.76	0.8853	0.3969
S4	8.31	8.01	6.95	0.81	0.4172	0.3751
S5	7.98	6.53	5.98	0.77	0.0939	0.6251
S6	8.52	6.88	5.35	0.77	0.0259	0.1889
S7	8.72	7.43	5.72	0.59	0.0120	0.0649
Withdrawal Period:						
W1	8.49	7.82	6.65	0.70	0.1656	0.2559
W2	8.83	8.80	6.62	0.57	0.0665	0.0744
W3	9.06	8.31	6.42	0.58	0.0358	0.0411
W4	8.98	8.54	6.94	0.60	0.1181	0.0849
Repeated Measures Analysis:						
	P value					
Treatment	0.0992					
Time	<0.0001					
Time x treatment	<0.0001					

² Contrast:

C vs D = Control vs the average of the two vitamin D₃ treatments.

2.5 vs 5.0 = 2.5 vs 5.0 MIU D₃ treatments.

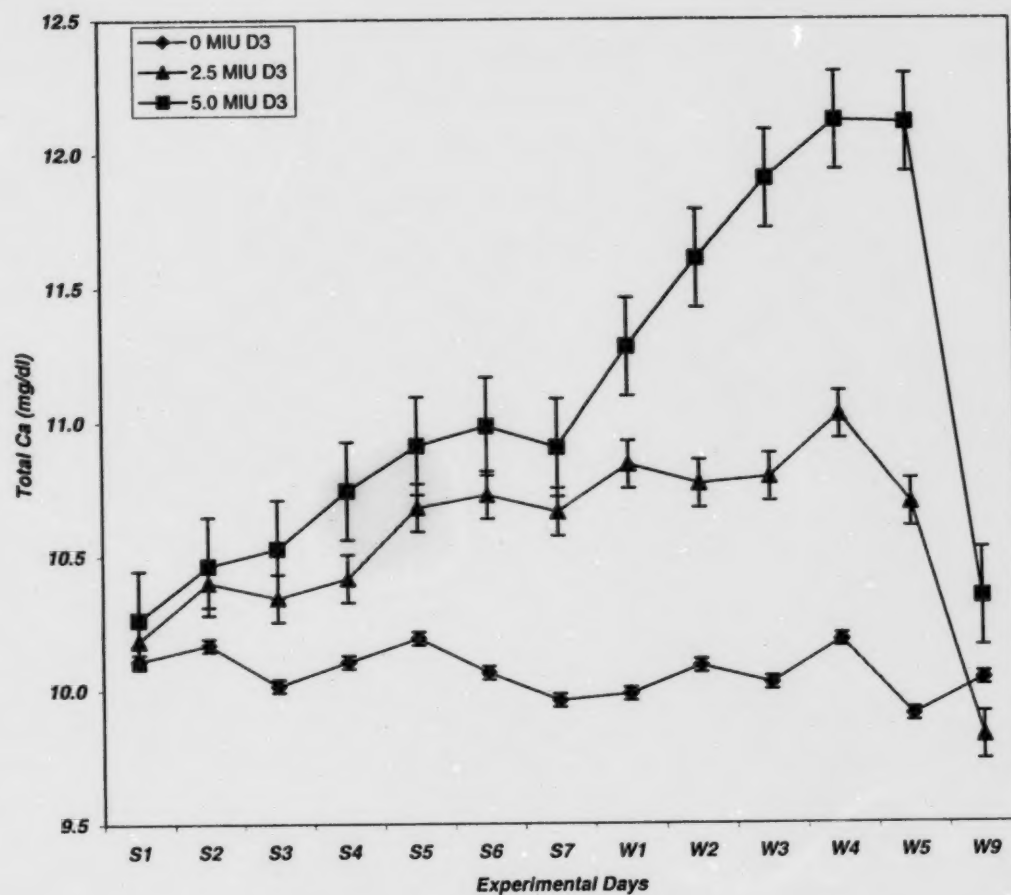


Figure 2.1. Effect of high levels of vitamin D₃ supplementation (0, 2.5 and 5 MIU per day for seven consecutive days) on total serum Ca concentrations of finishing Hereford steers (607 ± 12 kg BW), during adaptation (A), supplemental (S1 to S7) and withdrawal (W1 to W9) periods.

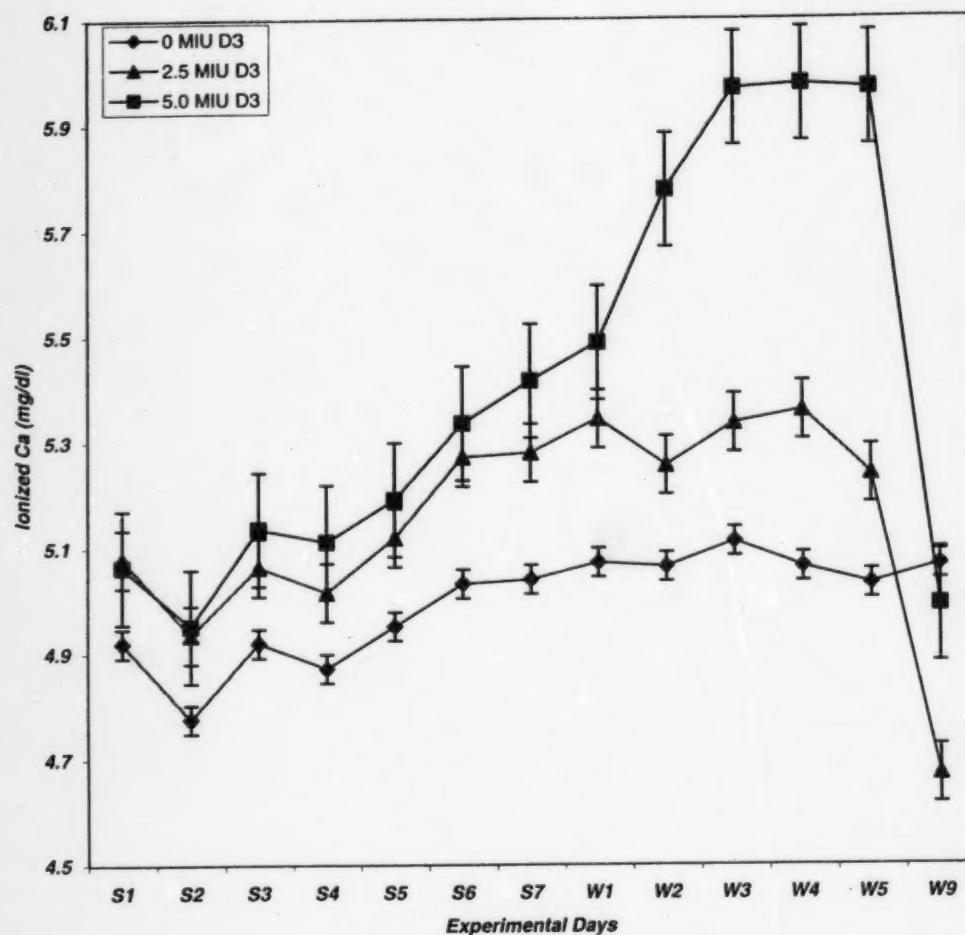


Figure 2.2. Effect of high levels of vitamin D₃ supplementation (0, 2.5 and 5 MIU per day for seven consecutive days) on ionized serum Ca concentrations of finishing Hereford steers (607 ± 12 kg BW), during adaptation (A), supplemental (S1 to S7) and withdrawal (W1 to W4) periods.

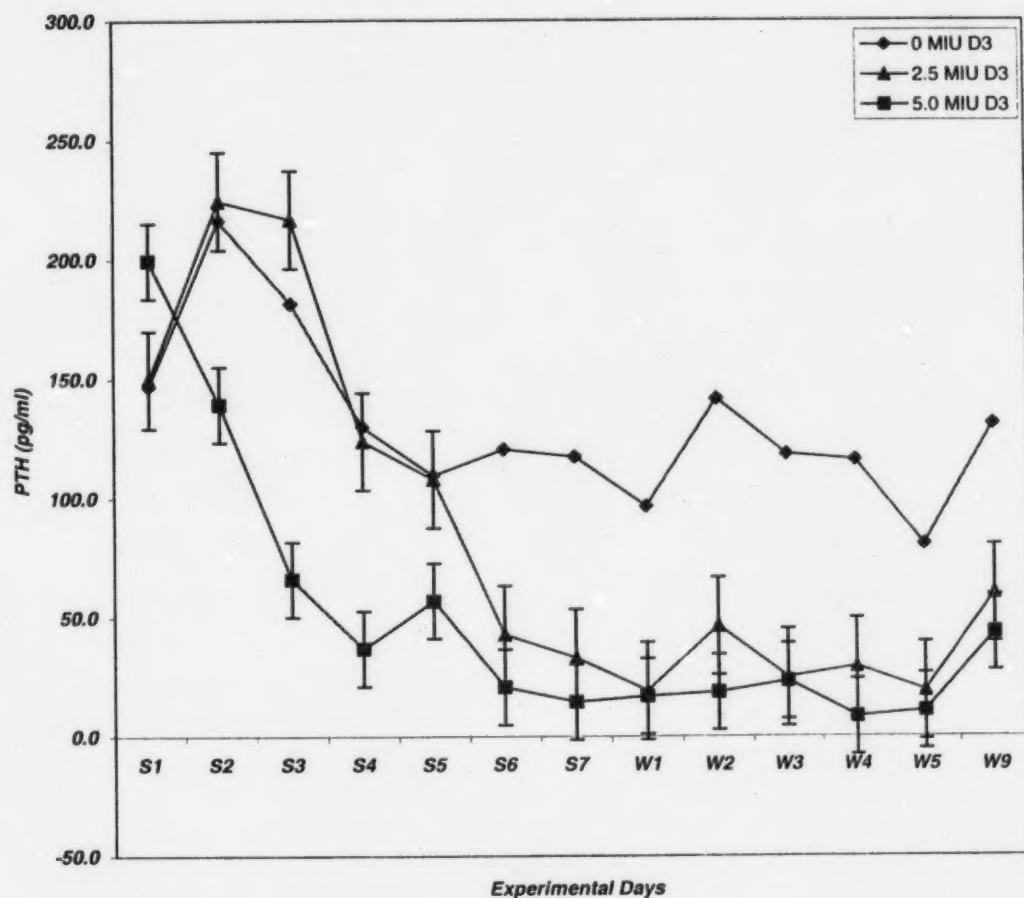


Figure 2.3. Effect of high levels of vitamin D₃ supplementation (0, 2.5 and 5 MIU for seven days) on serum parathyroid hormone (PTH) concentrations of finishing Hereford steers (607 ± 12 kg BW), during adaptation (A), supplemental (S1 to S7) and withdrawal (W1 to W4) periods.

Table 2.2. Effect of high levels of vitamin D₃ supplementation on serum calcitonin hormone concentrations of finishing Hereford steers (607 ± 12 kg BW).

Days	Calcitonin (pg/ml)					
	Vitamin D ₃ Level (MIU/hd/d)				Contrasts ^z	
	0	2.5	5.0	SEM	C vs D	2.5 vs 5.0
Supplemental Period:						
S1	354.02	381.94	297.64	15.94	0.4799	0.0028
S3	278.16	381.52	339.68	22.48	0.0112	0.2128
S6	296.36	323.34	352.68	23.27	0.1697	0.3902
Withdrawal Period:						
W1	318.62	309.08	281.72	30.86	0.5505	0.5425
W3	289.16	312.46	347.60	19.54	0.1133	0.2275
W5	332.04	310.42	328.08	22.99	0.6578	0.5970
Repeated Measures Analysis:						
	P value					
Treatment	0.4222					
Time	0.2246					
Time x treatment	0.0071					

^z Contrast:

C vs D = Control vs the average of the two vitamin D₃ treatments.

2.5 vs 5.0 = 2.5 vs 5.0 MIU D₃ treatments.

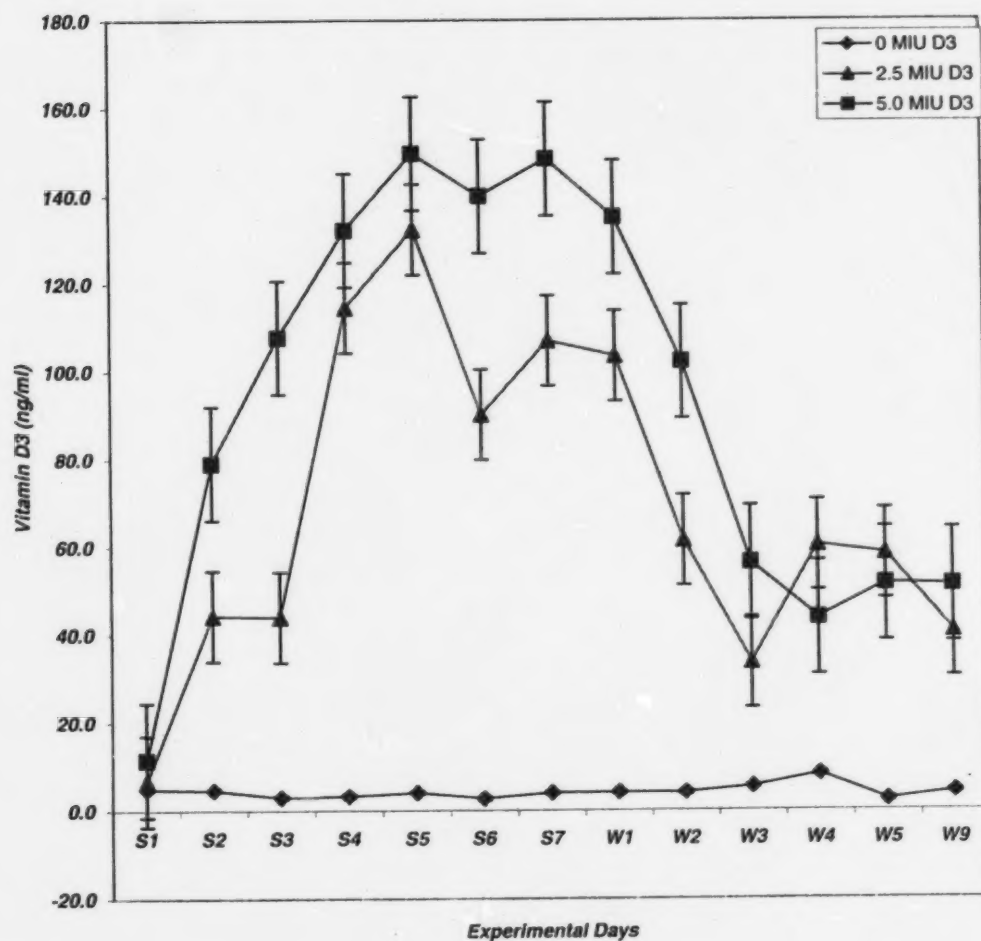


Figure 2.4. Effect of high levels of vitamin D₃ supplementation (0, 2.5 and 5 MIU for seven days) on plasma vitamin D₃ concentrations of finishing Hereford steers (607 ± 12 kg BW), during adaptation (A), supplemental (S1 to S7) and withdrawal (W1 to W4) periods.

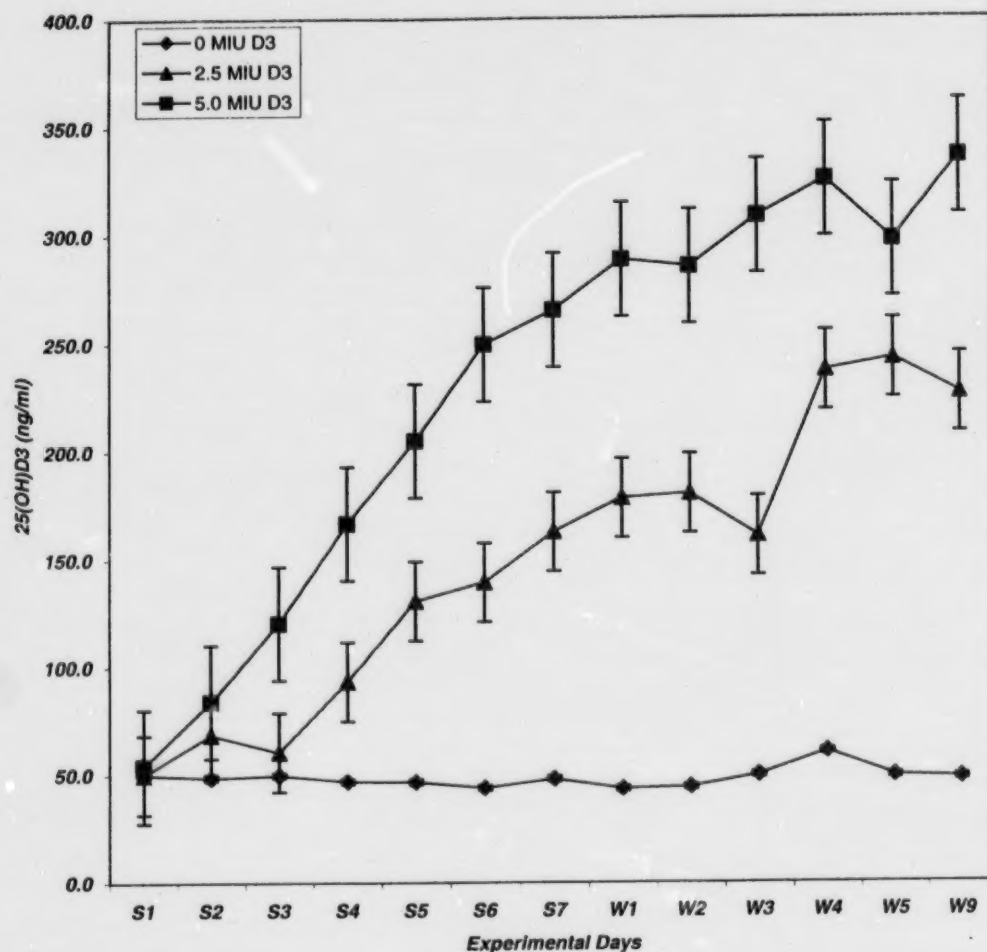


Figure 2.5. Effect of high levels of vitamin D₃ supplementation (0, 2.5 and 5 MIU for seven days) on plasma circulating 25-hydroxyvitamin D₃ (25(OH)D₃) concentrations of finishing Hereford steers (607 ± 12 kg BW), during adaptation (A), supplemental (S1 to S7) and withdrawal (W1 to W4) periods.

Experiment 3: Effect of Feeding a Low Calcium Diet and Vitamin D₃ on Calcium Metabolism in Finishing Steers.

Objective:

The objective of this study was to evaluate the feasibility of using a low Ca diet prior to vitamin D₃ supplementation at one of the three levels (1.25, 2.5 and 5 MIU per head per day for seven days) with the aim to increase serum Ca levels in finishing steers. It was also of interest to determine the effect of dietary treatment on parathyroid hormone, calcitonin, vitamin D₃, 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ levels in plasma.

Material and Methods:

Experimental Design:

Adaptation Period (A1 to A36):

Nineteen Angus steers (522 ± 34 kg BW) were housed in individual pens (3.6 x 3.6 m) with automatic water bowls in the Livestock Research Barn of the Department of Animal and Poultry Science at the University of Saskatchewan. Animals were cared for according to the guidelines of the Canadian Council of Animal Care. The cattle were adapted to the experimental diet for 36 days. The diet consisted of 75% concentrate (barley grain base) and 25% barley silage (as fed), meeting the requirements for this type of cattle according to the National Research Council (1996). The steers were fed twice a day (0800 and 1600 h), with orts collected and weighed every day before the morning feeding. Individual maximum voluntary feed intake was measured from day A30 to A36, constituting the pre-treatment feed intake value. The experimental steers were bled by jugular vein starting at 1230 h on the last day of this period (A36). Two sets of samples were taken. For the first, blood was collected into 5ml-draw evacuated (Vacutainer®) tubes without anticoagulant. The blood was allowed to clot at room temperature for approximately 2 h, then centrifuged at 3500 rpm for 15 min, the serum obtained was immediately used to determine total and ionized Ca concentrations. For the second set, blood was collected into 10 ml-draw evacuated (BD Vacutainer™) tubes with anticoagulant (sodium heparin), which were placed on crushed ice immediately after withdrawal, and approximately 2 h later centrifuged at 3000 rpm for 10 min at -4°C , then the plasma was collected and transferred to 1.7 ml centrifuge clear vials and stored at -20°C . These

plasma samples were used to determine vitamin D₃ and 25-hydroxyvitamin D₃ concentrations.

Low Ca Diet Period (L1 to L10):

The steers were randomly divided into four groups of five steers each. Groups 1, 2 and 3 were fed with a low Ca diet for ten days. This low Ca diet was essentially the same as the normal Ca diet but with the main source of Ca (limestone) withdrawn, resulting in a total Ca content of 0.14 % and a Ca to P ratio of 0.32, in contrast to 0.62 % and 1.43 for the normal Ca diet, respectively. Group 4 was fed the normal Ca (control) diet during this period. Feed was offered and intake monitored as in the previous period. Blood samples were obtained on days L2, L4, L6, L8 and L10 starting at 1230 h. These blood samples were collected, processed, stored and analyzed as described for the adaptation period.

Supplemental Period (S1 to S7):

All the steers were shifted to the normal Ca diet on day 11 (S1). The steers that received the low Ca diet (groups 1, 2 and 3) were then randomly assigned to one of the following levels of vitamin D₃ supplementation: 0, 1.25 and 2.5 million IU (MIU) D₃ per steer per day for seven consecutive days. The steers from group 4 (with no low Ca period) were supplemented with 5 MIU D₃ per steer per day for seven consecutive days. The vitamin D₃ supplement was prepared from a vitamin D₃ concentrate containing 500,000 IU per gram (Rhône-Poulenc Canada Inc.), using ground barley as a carrier. This supplement was formulated to have a final concentration of 13 MIU D₃ per kg of premix. Analysis of the supplement indicated an actual concentration of 14.06 ± 0.53 MIU D₃ per kg (Aventis Animal Nutrition, Inc. Alpharetta, GA. USA). The corresponding daily total amount of the vitamin D₃ supplement was equally divided and supplied at each feeding. The supplement was top-dressed onto the ration and thoroughly mixed by hand into the feed bunk. Feed intake was restricted to 85 % of the average voluntary intake during the last 7 days of the low Ca period with the exception of the control group (0 MIU) to ensure maximum vitamin D₃ consumption, with additional daily adjustments in order to avoid excessive feed refusals. Feed was supplied and feed intake monitored daily as described in previous

periods. Blood samples were obtained on day S2, S4 and S6, and these blood samples were collected, processed, stored and analyzed as described for the adaptation period.

Withdrawal Period (W1 to W11):

Once vitamin D₃ supplementation ended, the steers were maintained for a further six days under the experimental conditions to evaluate post-treatment effects, then the steers were shipped back to the feedlot. Feed was offered without restriction. The ration and intake monitoring were the same as in previous period. The steers were bled by jugular vein on days W1, W3, W5 and W11 (at the feedlot), these blood samples were collected, processed, stored and analyzed as described for the adaptation period. In addition, on the first day of the withdrawal period (W1) blood was collected using 10 ml-draw evacuated (BD Vacutainer™) tubes with anticoagulant (sodium heparin). Samples were placed in crushed ice immediately after withdrawal and approximately 2 h later centrifuged at 3000 rpm for 10 min at -4°C. Plasma was then transferred to 50 ml screw conical centrifuge tubes and stored at -20°C. These plasma samples were used to determine parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D₃ concentrations.

Laboratory Analyses:

Total and Ionized Serum Ca Determinations:

Serum samples were analyzed for total and ionized Ca as described in experiment 2 and 4. Plasma parathyroid hormone (PTH), calcitonin, vitamin D₃ and 25-hydroxyvitamin D₃ (25(OH)D₃) were as described in experiment 3. Plasma 1,25(OH)₂D₃ concentration was determined by radioimmunoassay (RIA)(1,25-Dihydroxyvitamin D ¹²⁵I RIA kit. DiaSorin, Stillwater, MN. USA).

Statistical Analysis:

The data was analyzed using the Analysis of Variance Technique including Repeated Measures Analysis using the General Lineal Model procedure of SAS (SAS 1989) to compare treatments within days and over time.

Contrast of interest included:

A = Normal Ca diet versus low Ca diet.

B = Control animals versus the average of vitamin D₃ treatments.

C = Between 1.25 and 2.5 MIU D₃ treatments.

D = Between 2.5 and 5 MIU D₃ treatments.

Results and Discussion:

Feed Intake (DMI):

Feed intake during the adaptation period averaged 9.67 ± 1.42 kg of DM, in which group 4 had a lower ($P < 0.05$) intake than the other three groups, an effect attributed to differences in live weight, as this group was lighter than the other three. During the low Ca period (ten days), groups 1, 2 and 3 were fed the low Ca diet (0.14 % Ca and 0.32 Ca to P ratio) and group 4 with the normal Ca diet (0.62 % Ca and 1.43 Ca to P ratio). Feeding the low Ca diet did not alter ($P > 0.05$) feed intake, with the exception of days L5 and L6 where intake was higher ($P < 0.05$) than the control group (4).

During the supplemental period (seven days), all the groups were shifted to the normal Ca diet. Groups 1, 2, 3 and 4 received 0, 1.25, 2.5 and 5 MIU D₃ per steer per day, respectively. Control animals (group 1) were fed *ad libitum*, while the vitamin D₃ supplemented steers were fed at 85% restriction, relative to the average intake from the previous period, and additionally daily adjustments were performed to avoid excessive refusals. There was no difference ($P > 0.05$) in feed intake among levels of vitamin D₃ supplemented.

During the withdrawal period (five days), the normal Ca diet continued to be fed to all steers but now without restriction. No differences ($P > 0.05$) among supplemented and control steers were found, except on the last day (W5), where consumption from control animals was higher than from vitamin D₃ supplemented steers. Similarly, there was a significant difference ($P < 0.05$) between the 1.25 and 2.5 MIU, but not between the 2.5 and 5 MIU treatments.

Minimum intakes were 6.37 (W1), 6.15 (S4), 5.23 (W3) and 3.90 (W3) kg, which represented a decrease in 10.8, 13.9, 26.8 and 45.4 % relative to the average intake on day 1 of supplementation, for the 0, 1.25, 2.5 and 5 MIU treatments, respectively. The

decline in intake from control animals (group 1) could be due to the handling of the steers for bleeding. Additional to this effect, supplementation of vitamin D₃ appeared to decrease feed intake, in a direct relationship to the amount supplemented.

The vitamin D₃ concentration of the supplement was 14.06 ± 0.53 MIU per kg (Aventis Animal Nutrition, Inc. Alpharetta, GA. USA). The supplement consumption averaged 1.27, 2.56 and 5.14 MIU D₃ per day for the 1.25, 2.5 and 5 MIU treatments, respectively. The higher concentration and better adjustment of the daily feed intakes helped to achieve the targeted consumption.

Total and Ionized Serum Ca Concentrations:

Serum total and ionized Ca concentrations in the adaptation period averaged 9.97 ± 0.26 and 4.90 ± 0.19 mg/dl, respectively. These concentrations and the ranges of 9.96 to 10.18 and 4.89 to 5.01 mg/dl for total and ionized Ca, respectively, for the control group during the low Ca period, were within the normal ranges reported for cattle.

Total Ca concentrations from the low Ca groups (1, 2 and 3) were lower ($P < 0.05$) than the control group (4), with the exception on days L4 and L10 (Fig. 3.1). Ionized Ca concentrations were also lower, with the exception on day L6 (Fig. 3.2).

During the first four first days of the supplemental period total and ionized Ca concentrations were not different ($P > 0.05$) between the control and supplemented steers or within levels of supplementation. However, at the end of this period (S6) total and ionized Ca from treated steers were higher ($P < 0.0001$ and $P = 0.0002$, respectively) than controls, and there were also differences ($P < 0.05$) within levels of supplementation. A response that was in direct relationship to the amount of vitamin D₃ supplemented. The ionized to total Ca ratio was not different between treated and control steers (Figs. 3.1 & 3.2). During the withdrawal period, total and ionized Ca concentrations continued rising, and were elevated ($P < 0.05$) relative to control animals, increases that followed the same pattern as in the previous period. However, these differences tended to disappear at the end of the withdrawal period (W11).

During the withdrawal period, maximal serum Ca values were achieved. Maximal concentrations for total Ca were 10.65, 11.50 and 12.41 mg/dl, which represented a 6.8, 15.3 and 24.5 % increase relative to control animals for the 1.25, 2.5 and 5 MIU

treatments, respectively, on day W3 (Fig. 3.1). Maximal concentrations for ionized Ca were 5.00, 5.57 and 6.03 mg/dl, which represented a 2.0, 13.7 and 23.1 % increase relative to control animals for the 1.25, 2.5 and 5 MIU treatments, respectively, on day W5 (Fig. 3.2).

The maximum serum Ca concentration of 12.41 mg/dl from the 5 MIU D₃ treatment was in good agreement with the range of results reported by other workers using the same level of supplementation and where improvements in beef tenderness were observed (Swanek *et al.* 1997, 1999; Montgomery *et al.* 1998, 2000; Karges *et al.* 1999b, 2001).

Comparing total and ionized serum Ca concentrations obtained in this experiment with previous experiments of this study, there was found a remarkable similarity among levels of supplementation. For example comparing study 1 with this study, total Ca: 11.67 vs 11.50 mg/dl and 12.43 vs 12.41 mg/dl for the 2.5 and 5 MIU treatments respectively. For ionized Ca: 5.73 vs 5.57 mg/dl and 6.03 vs 6.03 mg/dl for the 2.5 and 5 MIU treatments respectively.

Plasma Vitamin D₃ and 25-Hydroxyvitamin D₃ Concentrations:

Vitamin D₃ and 25(OH)D₃ plasma concentrations from the experimental steers during the adaptation period averaged 20.76 ± 7.00 and 23.22 ± 5.74 ng/ml, respectively. The vitamin D₃ pre-treatment average value and the range (13.43 to 30.68 ng/ml) from control animals during the low Ca period were slightly higher than the normal range (1 to 6 ng/ml) reported by DeLuca (1979) and Montgomery *et al.* (2000), however, this range was in good agreement with the values (<0.2 to 20 ng/ml) reported by Endres and Rude (2001). The 25(OH)D₃ pre-treatment value and the range (15.05 to 20.24 ng/ml) from control animals were in agreement with the normal range (25 to 35 or 10 to 50 ng/ml) reported in the literature (DeLuca 1979; Endres and Rude 2001, respectively).

During the low Ca diet period, vitamin D₃ concentration was decreased ($P < 0.0001$) in steers fed the low Ca diet relative to those fed the control diet during the first one to two days of this period. However, at the end of this period (L10), the situation was reversed (Fig. 3.3). The 25(OH)D₃ concentrations were similar between treated and untreated steers, with the exception of the last day of the period (L10), where the normal Ca cattle

had lower ($P < 0.05$) plasma $25(\text{OH})\text{D}_3$ concentrations than the low Ca diet groups (Fig. 3.4). These differences while significant were relatively small.

During the supplemental period, the supplemented animals showed an increase ($P < 0.0001$) in vitamin D_3 and $25(\text{OH})\text{D}_3$ concentrations. The response was closely related to the amount of vitamin D_3 supplemented ($P < 0.0001$). However, these two compounds had different response patterns. That is, the vitamin D_3 increase resembled a normal bell shaped curve, with the midpoint towards the end of the supplemental period. The $25(\text{OH})\text{D}_3$ levels exhibited a sustained linear increase until the beginning of the withdrawal period where plateaus were reached (Figs. 3.3 and 3.4).

Maximum plasma concentrations of vitamin D_3 were 103.25, 171.59 and 352.98 ng/ml, which represented a 5-, 8.2- and 13.1-fold increment relative to control animals for the 1.25, 2.5 and 5 MIU treatments, respectively. Maximum concentrations for $25(\text{OH})\text{D}_3$, were 91.40, 160.87 and 271.62 ng/ml, which represented a 5.6-, 10.2- and 17.2-fold increase relative to control animals for the 1.25, 2.5 and 5 MIU treatments, respectively (Figs. 3.3. and 3.4).

Montgomery *et al.* (2000) supplemented finishing steers with 5 MIU D_3 for 9 days and found at slaughter that plasma vitamin D_3 and $25(\text{OH})\text{D}_3$ concentrations reached 464.3 578.4 ng/ml, respectively. In the present study the increases in plasma of these compounds for the 5 MIU treatment were lower than the values reported by Montgomery *et al.* (2000). These workers, however, used a different method of vitamin D_3 administration (intraruminal boluses). Nevertheless, the response trends were similar between the two studies.

Plasma 1,25-Dihydroxyvitamin D_3 ($1,25(\text{OH})_2\text{D}_3$) Concentrations:

Plasma $1,25(\text{OH})_2\text{D}_3$ concentration was determined immediately after supplementation period ended (W1). The concentration was 26.83, 62.05, 113.99 and 148.71 pg/ml for the 0, 1.25, 2.5 and 5 MIU treatments, respectively, indicating a significant ($P < 0.0001$) increase in $1,25(\text{OH})_2\text{D}_3$ concentration with the level of vitamin D_3 supplementation (Table 3.1). Increases in $1,25(\text{OH})_2\text{D}_3$ concentration as a result of vitamin D_3 supplementation was to some extent contrary to what was expected as the second hydroxylation of vitamin D_3 in the kidney is a tightly regulated step. A possible explanation

for this phenomenon is that high levels of vitamin D₃ supplementation, induced high levels of vitamin D₃ and 25(OH)D₃ in plasma. It is possible that high substrate concentrations, mainly 25(OH)D₃, stimulated higher activity of the 1 α -hydroxylase enzyme, by overwhelming the negative feedback. This would in turn result in higher levels of 1,25(OH)₂D₃ and consequently higher levels of plasma Ca.

Plasma Parathyroid Hormone (PTH) Concentration:

Plasma PTH concentrations were determined immediately after supplementation period ended (W1). PTH concentrations were 74.65, 29.44, 12.51 and 5.00 pg/ml for the 0, 1.25, 2.5 and 5 MIU treatments, respectively. There was a significant ($P < 0.0001$) effect of treatment and between the lower and higher level of vitamin D₃ supplementation ($P < 0.05$), although the 2.5 MIU treatment was similar ($P > 0.05$) to both levels of supplementation (Table 3.1). PTH concentrations were in agreement with its theoretical response to high levels of plasma Ca.

Plasma Calcitonin Hormone Concentration:

The plasma calcitonin concentrations were 330.85, 384.72, 360.69 and 395.73 pg/ml for the 0, 1.25, 2.5 and 5 MIU treatments, respectively, with no effect ($P < 0.05$) of supplementation (Table 3.1). Calcitonin concentrations in this experiment were different to the theoretical trends expected. Calcitonin functions together with the other calciotropic hormones to maintain normal plasma Ca levels. When Ca concentrations are above normal, calcitonin is secreted immediately in order to prevent hypercalcemia (Copp 1973; Coles 1986; Guyton and Hall 1997). Even though, vitamin D₃ supplementation did increase serum Ca concentrations, calcitonin levels were not altered. According to Rossol *et al.* (1995) calcitonin regulatory mechanisms is considered weaker than the PTH system. This may explain why calcitonin levels were not significantly affected by vitamin D₃ supplementation.

In summary, feeding steers with the low Ca diet for ten days tended to decrease feed intake, plasma vitamin D₃, and total and ionized serum Ca concentrations but only for the first one to two days of feeding. Supplementing steers with vitamin D₃ for seven days resulted in a significant depression in intake, and in PTH levels, and in elevated levels of

total and ionized serum Ca, as well as in plasma vitamin D₃, 25(OH)D₃, and 1,25(OH)₂D₃ levels, with no effect on calcitonin levels.

Total serum Ca concentrations were increased by almost 0.5 mg/dl more (11.50 vs 11.02 mg/dl) than in the previous experiment (Chapter IV) for the 2.5 MIU treatment. It is possible to theorize that the increased response in the present study for the same level of vitamin D₃ supplementation was a result of the low Ca diet, which may have primed the animal to respond to vitamin D₃ supplementation. However, it is also possible that the improved response is in relation to differences in the actual consumption of vitamin D₃. In the previous experiment, the steers fed the 2.5 MIU D₃ level consumed 84 % (2.1 MIU/d) of the total amount targeted, while in the present trial the consumption was slightly higher than that targeted (2.7 MIU/d). In the previous study steers fed the 5 MIU D₃ level consumed approximately 70 % (3.4 MIU/d) of the total amount targeted, while in this experiment consumption was almost exactly that targeted (5.1 MIU/d).

An interesting finding of this study was the fact that plasma 1,25(OH)₂D₃ concentrations were increased as a result of vitamin D₃ supplementation, contrary to the physiological normal response.

The apparent lack of response of the low Ca diet during the feeding period or in enhancing the effect of vitamin D₃ on serum Ca concentrations may be due to the following factors. First, low Ca diets in dairy cattle are fed for long periods of time (30 to 80 days); and secondly, and most importantly, low Ca diets usually do not increase plasma Ca levels during the feeding period, but they prevent sharp drops of plasma Ca levels at parturition and/or initiation of lactation, by increasing Ca resorption from bone and the efficiency of Ca absorption from the intestine. Through stimulation of the synthesis of PTH and 1,25(OH)₂D₃, these diets actually prime the Ca homeostatic mechanisms of the animal. In this trial, feeding the low Ca diet altered the Ca homeostatic mechanisms as initial levels of total Ca and vitamin D₃ were initially lower than normal. However, the lack of any major stress, as parturition imposes to the high-producing cow seems to neglect any advantage from the low Ca diet prior to supplementation of vitamin D₃. It does not appear that feeding a low Ca diet to finishing steers enhances the serum Ca response of the animals to vitamin D₃ feeding.

Conclusions:

Feeding a low Ca diet for ten days prior to vitamin D₃ supplementation induced a decrease in feed intake, total serum Ca and plasma vitamin D₃ concentrations during the first three to four days of feeding, but had no effect on other parameters.

High levels of vitamin D₃ (1.25, 2.5 and 5 MIU per steer per day for seven consecutive days) had a significant effect on depressing feed intake and plasma PTH levels. On the other hand, it significantly increased total and ionized serum Ca, and plasma vitamin D₃, 25(OH)D₃ and 1,25(OH)₂D₃ concentrations. The degree of response was intimately related to the levels of vitamin D₃ supplementation. However, no effect on the ionized to total Ca ratio or in calcitonin concentrations were observed.

An interesting finding from this experiment is that supplementation with high levels of vitamin D₃ actually increases the plasma concentration of 1,25(OH)₂D₃ despite high concentrations of total and ionized Ca in serum. This phenomenon has been also observed in dairy cows when they have been injected with high amounts of vitamin D₃. These results suggest that in ruminants the activity of the enzyme 1 α -hydroxylase is not as tightly regulated as is in humans and other animals (pigs).

It seemed that the low Ca diet affected the Ca homeostatic mechanisms at least to some degree because total Ca and vitamin D₃ concentrations in blood were lower than normal during the first days of the low Ca period. However, the lack of any stress such as parturition appears to counteract the potential benefits of feeding a low Ca diet to finishing steers.

In conclusion, it does not appear that feeding a low Ca diet to finishing steers enhances the serum Ca response of the animals to vitamin D₃ feeding.

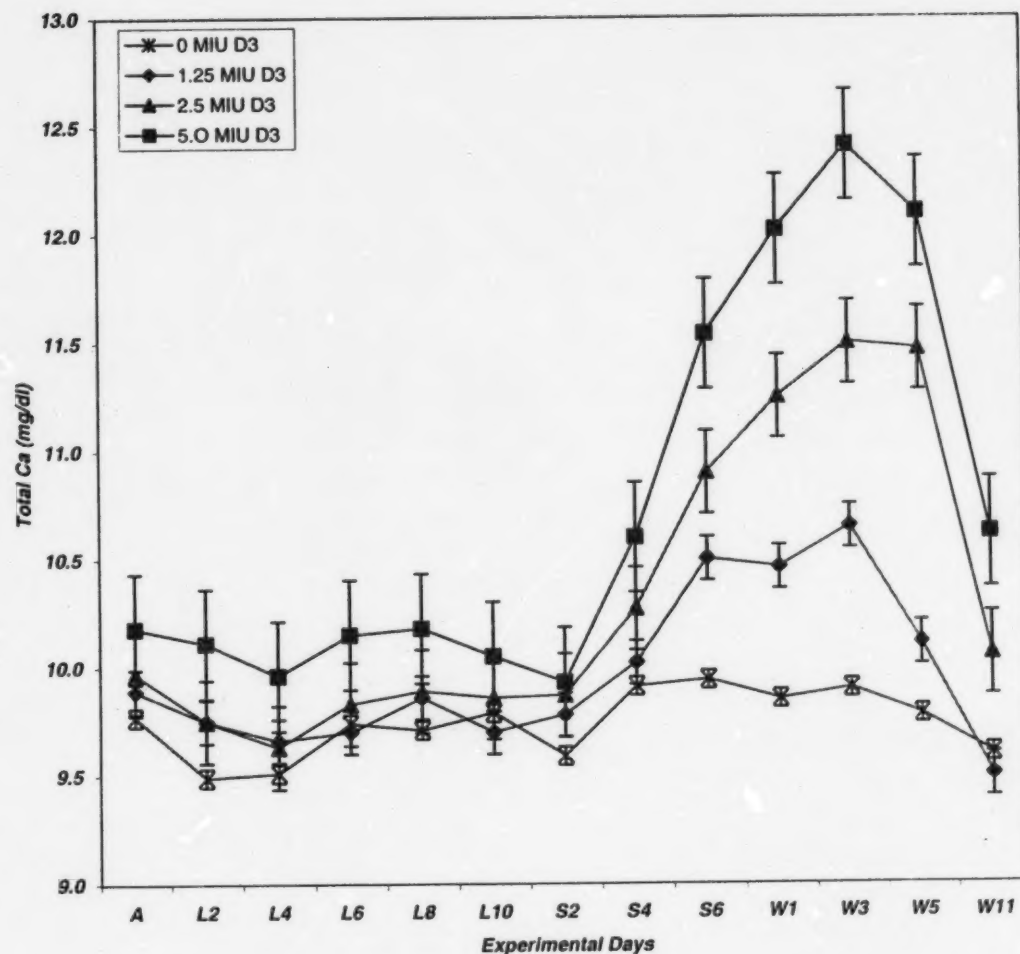


Figure 3.1. Effect of feeding a low Ca diet for ten days prior to supplementation of vitamin D₃ (0, 1.25, 2.5 and 5 MIU per day for seven consecutive days) on total serum Ca concentrations of finishing Angus steers (522 ± 33 kg BW), during adaptation (A), low Ca (L1 to L10), supplemental (S1 to S7) and withdrawal (W1 to W11) periods.

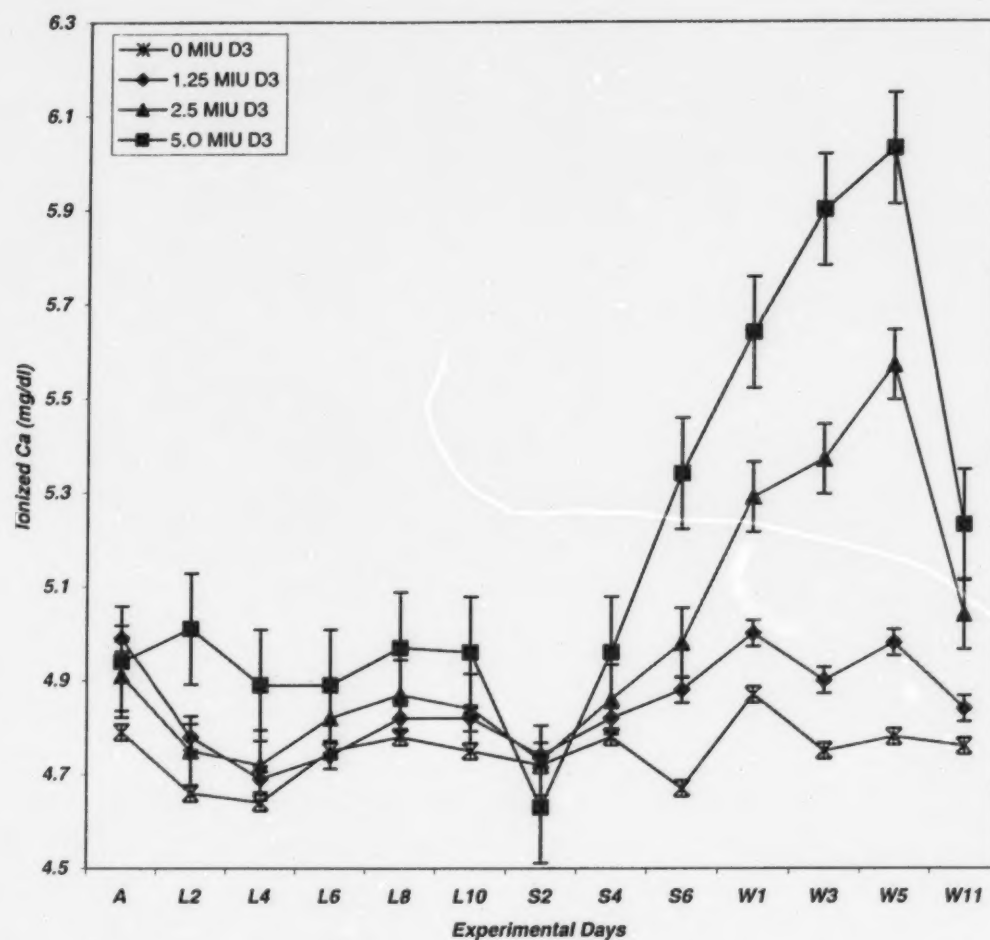


Figure 3.2. Effect of feeding a low Ca diet for ten days prior to supplementation of vitamin D₃ (0, 1.25, 2.5 and 5 MIU per day for seven consecutive days) on ionized serum Ca concentrations of finishing Angus steers (522 ± 33 kg BW), during adaptation (A), low Ca (L1 to L10), supplemental (S1 to S7) and withdrawal (W1 to W11) periods.

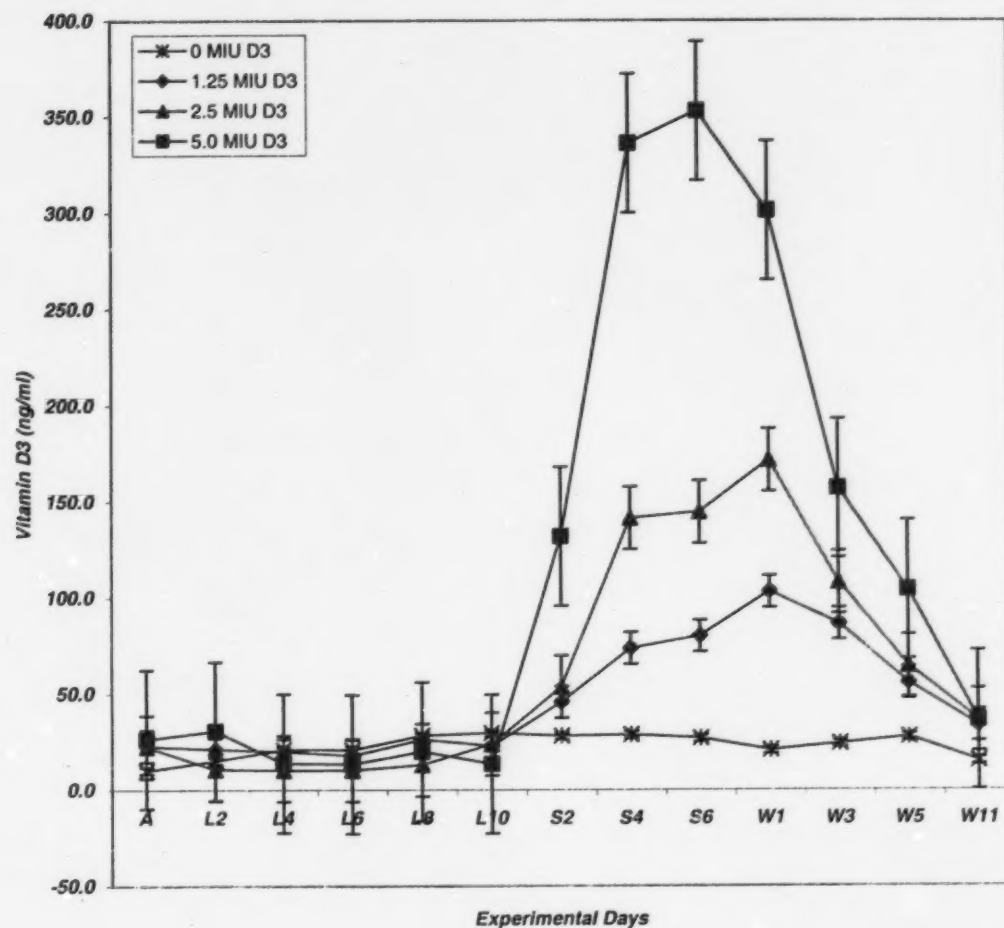


Figure 3.3. Effect of feeding a low Ca diet for ten days prior to supplementation of vitamin D₃ (0, 1.25, 2.5 and 5 MIU per day for seven consecutive days) on plasma vitamin D₃ concentrations of finishing Angus steers (522 ± 33 kg BW), during adaptation (A), low Ca (L1 to L10), supplemental (S1 to S7) and withdrawal (W1 to W11) periods.

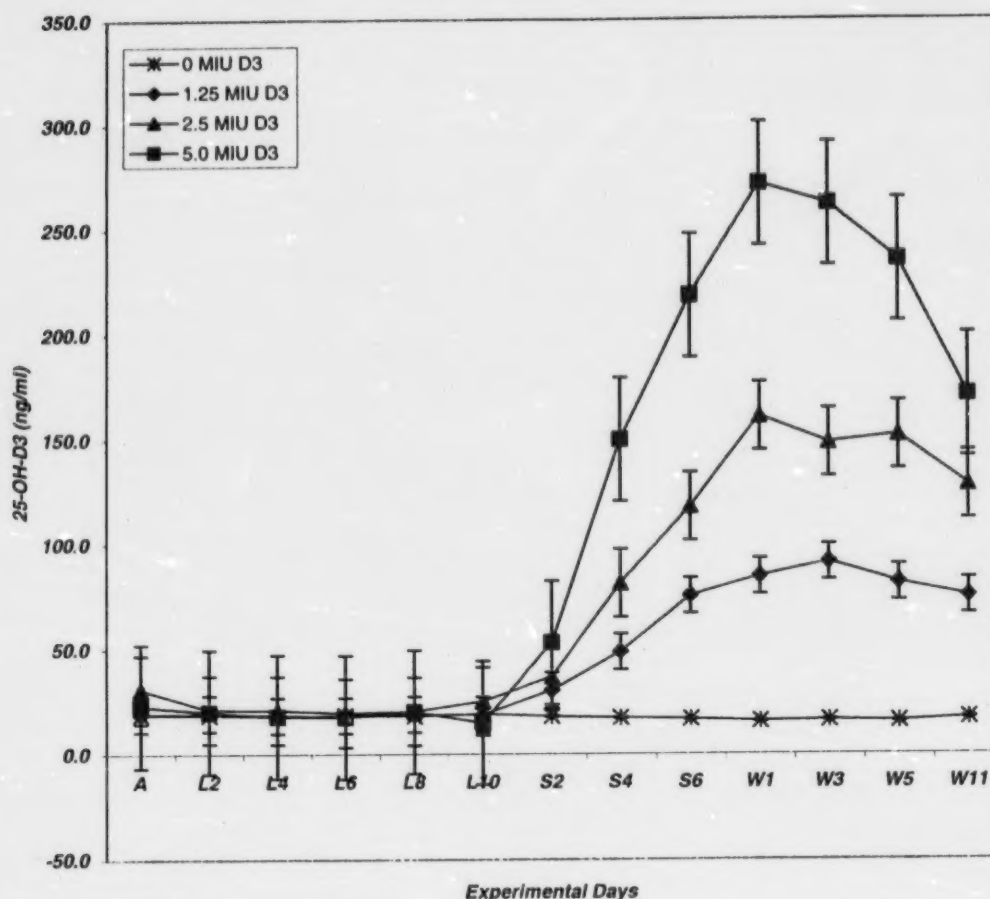


Figure 3.4. Effect of feeding a low Ca diet for ten days prior to supplementation of vitamin D₃ (0, 1.25, 2.5 and 5 MIU per day for seven consecutive days) on 25-hydroxyvitamin D₃ (25(OH)D₃) concentrations of finishing Angus steers (522 ± 33 kg BW), during adaptation (A), low Ca (L1 to L10), supplemental (S1 to S7) and withdrawal (W1 to W11) periods.

Table 3.1. Effect of feeding a low Ca diet for ten days prior to supplementation of vitamin D₃ on circulating calciotropic hormones concentration of finishing Angus steers (522 ± 33 kg BW) on day 1 of the withdrawal period.

Calciotropic Hormones (pg/ml)								
Day	Vitamin D ₃ Level (MIU/hd/d)					Contrast ²		
	0	1.25	2.5	5.0	SEM	A	B	C
Parathyroid Hormone (PTH):								
W1	74.65	29.44	12.51	5.00	6.40	<0.0001	0.0552	0.3964
1,25-Dihydroxyvitamin D ₃ :								
W1	26.83	62.05	113.99	148.71	12.25	<0.0001	0.0047	0.0531
Calcitonin:								
W1	330.86	384.72	360.69	395.73	29.93	0.1671	0.5358	0.3976

^a Contrasts:

A = Control animals (0 MIU) versus supplemented vitamin D₃ steers.

B = Treatment 1.25 versus 2.5 MIU D₃.

C = Treatment 2.5 vs 5 MIU D₃.

Experiment 4: Effect of Low and High Calcium Diets, Anionic Salts and Vitamin D₃ Supplementation to Finishing Beef Steers on Calcium and Acid-Base Homeostasis.

Objectives:

The objective of this experiment was to evaluate the use of a low Ca diet for fourteen days prior to feeding a high Ca diet supplemented with anionic salts (1500 and then 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU) for ten days on plasma total and ionized Ca concentrations and on calcium and acid – base homeostasis of the animal.

Materials and Methods:

Experimental Design:

Adaptation Period (A1 to A19):

Twenty Hereford steers (448 ± 26.2 kg BW) were housed in individual pens (3.6 x 3.6 m) with automatic water bowls in the Livestock Research Barn of the Department of Animal and Poultry Science at the University of Saskatchewan. Animals were cared for according to the guidelines of the Canadian Council of Animal Care. The cattle were adapted to the experimental diet for 19 days. The diet consisted of 75 % concentrate (barley grain based) and 25 % barley silage (as fed). This diet was considered a normal Ca diet and was formulated according to the Nutrient Requirements of Beef Cattle (NRC 1996), which had a total Ca concentration of 0.64 % and a Ca to P ratio of 1.3 (Enviro-Test Laboratories, Saskatoon, SK, Canada and Norwest Labs, Lethbridge, AB, Canada). The dietary cation-anion balance (DCAB) $([Na+K] - [Cl+S])$ for this diet was 2.07 mEq/kg of DM (Oetzel 1993).

The steers were fed twice a day (0800 and 1600 h), with orts collected and weighed before the morning feeding. Individual maximum voluntary feed intake was measured from day A13 to A19, constituting the pre-treatment feed intake value.

The experimental steers were bled by jugular vein starting at 1230 h on the last day of this period (A19). Four sets of samples were taken for analysis of total and ionized Ca concentrations; and parathyroid hormone, calcitonin and 1,25-dihydroxyvitamin levels as well as for vitamin D₃ and 25-hydroxyvitamin D₃ concentrations and acid base parameters

(pH, HCO_3 , base excess) using a blood gas analyzer (Radiometer 288 System, Copenhagen, Denmark).

Low Ca Diet Period (L1 to L14):

The steers were randomly divided into four groups (five steers per group) and fed with a low Ca diet for fourteen days. The total ration consisted of 75 % concentrate (barley grain based) and 25 % barley silage (as fed). However, the source of supplemental Ca (limestone) from the concentrate was withdrawn. The chemical analysis (Enviro-Test Laboratories, Saskatoon, SK, Canada and Norwest Labs, Lethbridge, AB, Canada) indicated that the complete ration had a total Ca concentration of 0.16 % and a Ca to P ratio of 0.31. The DCAB for this diet was 1.77 mEq/kg of DM (Oetzel 1993). The feeding pattern and the monitoring of the feed intake were the same as in the previous period. Blood samples from all the steers were obtained on days L7 and L14, which were collected, processed, stored and analyzed as described for the adaptation period.

Supplemental Period (S1 to S10):

Immediately after the low Ca diet period ended, all the four groups of steers were switched to a high Ca diet. The total ration consisted of 75 % concentrate (barley grain based) and 25 % barley silage (as fed). The chemical analysis (Enviro-Test Laboratories, Saskatoon, SK, Canada and Norwest Labs, Lethbridge, AB, Canada) indicated that the complete ration had a total Ca concentration of 0.84 % and a Ca to P ratio of 1.68.

The DCAB for this diet: 2.19 mEq/kg of DM was calculated following to the guidelines provided by Oetzel (1993), according to the chemical analysis (Norwest Labs, Lethbridge, AB, Canada) of the ration. The feeding pattern and the monitoring of feed intake were the same as in previous period. During this period, all four groups of steers were supplemented with anionic salts. The anionic salts supplemented included magnesium sulfate (MgSO_4) and ammonium chloride (NH_4Cl). The addition of these salts was such that the DCAB was brought down to - 1500 mEq/d. This was accomplished by adding 56.57 g of MgSO_4 and 56.57 g of NH_4Cl per day for three days (S1 to S3). For the remaining seven days of the supplemental period (S4 to S10) the DCAB was reduced to - 3000 mEq/d by adding 113.5 g of MgSO_4 and 113.5 g of NH_4Cl per day. The total amount

of these minerals were equally divided and offered at each feeding by top-dressing the ration and then mixing it thoroughly by hand into the feed bunk. Additionally, 50 mg of thiamin were added to the ration in the morning feeding. In addition, groups 1, 2, 3 and 4 were assigned to the following levels of vitamin D₃ supplementation: 0, 0.6, 1.2 and 2.4 million IU (MIU) of vitamin D₃ (D₃) per steer per day for ten consecutive days, respectively.

The vitamin D₃ supplement was prepared from a vitamin D₃ concentrate containing 518,000 IU per gram (Aventis Animal Nutrition Canada Inc. Campbellville, Ontario), using ground barley as a carrier. This supplement was formulated to have a final concentration of 13 MIU D₃ per kg of premix. Analysis of the supplement indicated an actual concentration of 12.66 ± 0.21 MIU D₃ per kg (CN Laboratories. Courtland, MN. USA).

Feed intake was restricted to 85% relative to the average consumption from the last seven days of the low Ca diet period in all the steers, and additional daily adjustments were carried out to avoid excessive feed refusals and so induce maximum ionic salt and vitamin D₃ consumption. Feeding times and feed intake monitoring were as described in previous periods. Blood samples from all the steers were obtained on days S1, S3, S5, S7, and S9. These blood samples were collected, processed, stored and analyzed as described in the adaptation period.

Withdrawal Period (W):

Once ionic salts and vitamin D₃ supplementation ended, the steers were maintained for a further five days under the experimental conditions to evaluate post-treatment effects. The steers were maintained on the same basal diet *ad libitum*. Feeding pattern and feed intake monitoring were as described in previous periods.

Blood samples from all the steers were obtained on days W1 and W5. These blood samples were collected, processed, stored and analyzed as described in the adaptation period.

Laboratory Analyses:

Blood samples were analyzed for total and ionized Ca, vitamin D₃, 25(OH)D₃, 1,25(OH)₂D₃, PTH and calcitonin levels as described in previous experiments. Blood gas analysis was carried out by using the Radiometer 288 System (Radiometer 288 System,

Copenhagen, Denmark). The analysis included pH, partial pressure of carbon dioxide ($p\text{CO}_2$), total carbon dioxide ($t\text{CO}_2$), partial pressure of oxygen ($p\text{O}_2$), bicarbonate (HCO_3^-), base excess (BE), hemoglobin (tHb), sodium (Na), potassium (K), chloride (Cl) and anion gap (AG).

Statistical Analysis:

The data from feed intake and blood sample analyses were analyzed using the Analysis of Variance Technique including Repeated Measures Analysis using the General Lineal Model procedure of SAS (SAS 1989) to compare treatments within days and over time. **Contrasts of interest included:**

- 1) Pre-treatment control value versus low Ca groups.
- 2) Control animals versus vitamin D_3 supplemented steers.
- 3) Treatment 0.6 versus 1.2 MIU D_3/d .
- 4) Treatment 1.2 versus 2.4 MIU D_3/d .

Results:

Feed Intake (DMI):

Feed intake during the adaptation period averaged 9.93 ± 1.16 kg DM. Feed intake was not affected ($P < 0.05$) during the fourteen days of feeding the low Ca diet. During the supplementation period (anionic salts and vitamin D_3) the high Ca diet was fed. Feed was offered at 85 % of that consumed during the last seven days of the previous period. During this period the high Ca diet was supplemented with anionic salts at - 1500 mEq/d for the first three days. Subsequently, the addition of anionic salts was increased to - 3000 mEq/d for the next seven days. In order to evaluate the effect of anionic salt supplementation, the average feed intake (restricted to 85% of *ad libitum*) during the low Ca period was contrasted with the intake of the steers supplemented only with anionic salts (group 1). Anionic salts did not decrease ($P < 0.05$) feed intake of control animals during the first seven days of supplementation. However, feed intake was decreased ($P < 0.05$) during the last three days of this period. Feed intake of control steers was depressed by 26.1 % relative to pre-supplementation of anionic salts on day S10.

During the first three days of supplementation feed intake was no different ($P>0.05$) between anionic and vitamin D₃ supplemented steers or between levels of vitamin D₃ supplementation. From the fourth day of supplementation anionic salts were increased to -3000 mEq/d, and feed intake started to decrease. The pattern of decline was similar to all groups of steers. The last three days of supplementation, there was a significant difference ($P<0.05$) between control steers (anionic salts only) and vitamin D₃ supplemented steers. However, there was not difference ($P>0.05$) between vitamin D₃ groups. Even though, there was a tendency to decrease feed intake in an inverse relationship to the amount of vitamin D₃ supplemented (Fig. 4.1). On the last day of this period (S10), feed intake in all groups reached their minimum values, which were 6.19, 5.57, 3.84 and 4.04 kg. Immediately after anionic salt supplementation was withdrawn, feed intake started to recover for all groups (Fig. 4.1). The feed intake levels for groups 1 and 2 (anionic salts only and 0.6 MIU D₃, respectively) returned to pre-treatment values by the last day of the withdrawal period (W4). However, feed intake did not return to pre-treatment levels for groups 3 and 4 (1.2 and 2.4 MIU treatments, respectively) even four days after withdrawal.

The average consumption of the vitamin D₃ supplement was 94.3 % (0.55 MIU/d), 87.8 % (1.0 MIU/d) and 88.92 % (2.1 MIU/d) of the targeted intake for the 0.6, 1.2 and 2.4 MIU treatments, respectively.

Total and Ionized Ca Concentrations:

Total and ionized serum Ca concentrations during the adaptation period averaged 9.81 ± 0.34 and 4.99 ± 0.19 mg/dl, respectively. These averages were not affected ($P>0.05$) by feeding the low Ca diet, with the exception of group 1 and 4 that had lower ($P<0.05$) ionized and total Ca concentrations on day L14, respectively. In order to know the effect of anionic salts on total and ionized serum Ca, the average concentrations from the low Ca period were contrasted with the measurements for the group of animals supplemented with anionic salts only (group 1). Anionic salts increased total Ca ($P<0.05$) and ionized Ca ($P<0.0001$) concentrations during the supplemental and withdrawal periods, with the exception of day S1 (total Ca) and W5 (ionized Ca) (Figs. 4.2 and 4.3).

During the first three days of supplementation (- 1500 mEq/d and 0, 0.6, 1.2 and 2.4 MIU D₃/d), total and ionized serum Ca concentrations began to increase ($P < 0.0001$), with no difference ($P > 0.05$) between the control and vitamin D₃ treated animals (Figs 4.2 and 4.3). From day S4 to S10, anionic salts were increased to 3000 mEq/d with vitamin D₃ supplementation at the same levels. Total and ionized Ca continued rising ($P < 0.0001$) in all four groups. A clear differentiation ($P < 0.05$) between control and the vitamin D₃ treatment was found, but not within levels of vitamin D₃ ($P > 0.05$). Total and ionized serum Ca for the 0, 0.6, 1.2 and 2.4 MIU treatments tended to reach a plateau towards the end of the supplemental period. However, the 2.4 MIU treatment induced ($P < 0.0001$) maximum total and ionized Ca concentrations during the withdrawal period, where vitamin D₃ treated animals still had higher ($P < 0.05$) values than the control animals (Figs. 4.2 and 4.3). Maximum total serum Ca concentrations achieved were 10.11 (S7), 10.97 (W1), 11.43 (S7) and 12.24 (W1) mg/dl for the 0, 0.6, 1.2 and 2.4 MIU D₃ treatments, respectively. This represents a 3.1, 11.8, 16.5 and 24.8 % increase relative to the pre-treatment control value, respectively (Fig. 4.2). Maximum ionized serum Ca concentrations were 5.43 (S7), 5.90 (W1), 5.98 (S7) and 6.25 (W1) mg/dl for the 0, 0.6, 1.2 and 2.4 MIU D₃ treatments, respectively. This represents an 8.8, 18.2, 19.8 and 30.7 % increase relative to the pre-treatment control value, respectively (Fig. 4.3).

Vitamin D₃ and 25-Hydroxyvitamin D₃ (25(OH)D₃):

Vitamin D₃ plasma concentrations during the adaptation period averaged 16.81 ± 5.04 ng/ml. The low Ca diet decreased ($P < 0.05$) vitamin D₃ plasma concentration in all groups on day L7, however, concentrations returned to normal by day L14. Anionic salt supplementation at levels of 1500 mEq/d decreased ($P < 0.05$) plasma vitamin D₃ concentrations, although at 3000 mEq/d there was no effect ($P > 0.05$), with the exception on day S7. However, during the withdrawal period, vitamin D₃ levels were higher ($P < 0.0001$) in cattle that were fed only the anionic salts relative to values for the low calcium period.

Plasma vitamin D₃ concentrations were not different ($P > 0.05$) between steers supplemented with anionic salts or anionic salts plus vitamin D₃, irrespective of the level of vitamin D₃ fed, on day S1. However, plasma vitamin D₃ concentrations increased

($P < 0.0001$) in steers supplemented with vitamin D_3 from day S3 to W5, with clear differentiation ($P < 0.05$) between levels of vitamin D_3 supplementation (Fig. 4.4). Plasma vitamin D_3 concentration response curves described a bell shaped curve, with the degree of response directly related to the level of vitamin D_3 supplemented. The response was observed almost immediately after supplementation initiated and returned almost to normal by the end of the withdrawal period. However, at this point the concentrations for all treated animals (including anionic salts only) were still higher ($P < 0.05$) than pre-treatment values. Maximum vitamin D_3 plasma concentration achieved for the 0, 0.6, 1.2 and 2.4 MIU treatments were 28.31 (W5), 50.13 (W1), 78.24 (S5) and 122.55 (S5) ng/ml, respectively, which represented a 1.7-, 3.0-, 4.6- and 7.3-fold increase relative to pre-treatment values (Fig. 4.4).

Plasma 25(OH) D_3 concentrations during the adaptation period averaged 20.77 ± 3.39 ng/ml. This average was not affected ($P > 0.05$) by the low Ca diet. Anionic salt supplementation decreased ($P < 0.05$) the concentrations of 25(OH) D_3 during the first five to six days of supplementation and again on the first day of the withdrawal period (W1). Supplementation of anionic salts plus vitamin D_3 induced sustained ($P < 0.0001$) increases in plasma 25(OH) D_3 that started to rise almost immediately after supplementation began, reaching maximum values towards the end of the withdrawal period. There were large differences ($P < 0.0001$) in 25(OH) D_3 concentrations between anionic salts and vitamin D_3 supplemented steers. There was clear ($P < 0.0005$) differentiation between the vitamin D_3 treatments during the supplemental and withdrawal periods. The maximum 25(OH) D_3 concentrations for 0.6, 1.2 and 2.4 MIU treatments were 63.81 (W1), 97.34 (W1) and 154.54 (W5) ng/ml, respectively, which represented a 3.9-, 5.9- and 9.4-fold increase relative to the control group (Fig. 4.5).

Calcitrophic Hormones:

Serum concentrations of the parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 and calcitonin were determined immediately after supplementation of anionic salts and vitamin D_3 ended (W1). Supplementation of vitamin D_3 decreased ($P < 0.0001$) PTH values, and there also was a difference ($P < 0.05$) between the 0.6 and 1.2 MIU D_3 treatments, but no between the 1.2 and 2.4 MIU D_3 treatments (Table 4.1). 1,25(OH) $_2D_3$ concentrations

were increased ($P < 0.001$) as a result of vitamin D₃ supplementation, although there was not difference ($P > 0.05$) between vitamin D₃ treatments (Table 4.1). Calcitonin concentrations were not significantly altered ($P > 0.05$) as a consequence of vitamin D₃ supplementation (Table 4.1).

Acid-Base Status:

Blood pH values during the adaptation period averaged 7.40 ± 0.02 . During the low Ca diet blood pH was decreased ($P < 0.05$) in all groups on day L14. Anionic salt supplementation at the level of 1500 mEq/d did not affect ($P > 0.05$) blood pH values. However, feeding the anionic salts at 3000 mEq/d reduced ($P < 0.0001$) blood pH. This effect was still evident at the beginning of the withdrawal period. The depressing effect of anionic salts on pH was such that when ionic salts were withdrawn, pH values returned slightly higher ($P < 0.05$) than normal on day W5. There was no difference ($P > 0.05$) in pH values between cattle fed anionic salts and those fed anionic salts plus vitamin D₃, nor between levels of vitamin D₃. The repeated measures analysis showed a highly significant effect of time ($P < 0.0001$), but not treatment ($P = 0.7014$) or time by treatment interaction ($P = 0.6959$). Indicating that pH values were effectively deviated from normal as a result of anionic salt supplementation (Fig. 4.6).

This same pattern of response for blood pH values to anionic salt supplementation was observed for total carbon dioxide (tCO₂), bicarbonate (HCO₃⁻), and base excess (BE) values. However, partial pressure of carbon dioxide (pCO₂), partial pressure of oxygen (pO₂) and anionic gap (AG) were not significantly altered ($P < 0.05$) at any level of anionic salt or vitamin D₃ treatment (data not shown).

Discussion:

The main objective of this experiment was to determine if it is possible to enhance the effect of vitamin D₃ supplementation on increasing total and ionized serum Ca concentrations through the manipulation of dietary Ca and the feeding of anionic salts. A low Ca diet was fed initially for fourteen days prior to supplementation of vitamin D₃. In the previous experiment it was found that the feeding a low Ca diet decreased total and ionized serum Ca as well as plasma vitamin D₃ concentrations during the first days of

feeding. It was concluded in this study that the low Ca diet might have primed the Ca homeostatic mechanism in the steers. For this reason, the same approach was used in this experiment. It has also been shown that the acidification of the diet through the addition of anionic salts cause hypercalciuria (Moore 1970; Horst *et al.* 1997). This phenomenon also activates the Ca homeostatic mechanisms, principally by increasing the efficiency of intestinal Ca absorption. For this reason, a ration high in Ca was fed while supplementation of anionic salts and vitamin D₃ was carried out. On the other hand, anionic salts appear to be unpalatable and can lead to a depression in intake. For this reason it should be added gradually to the ration. Thus, supplementation of anionic salts was done in two steps: at 1500 mEq/d for three days and then at 3000 mEq/d for the rest of the trial (seven days).

Feed intake in this experiment was depressed as a result of anionic salt supplementation more than due to vitamin D₃ supplementation. However, the effect was only significant at the highest level of supplementation (3000 mEq/d). Although not significant, the decline in intake followed a trend that was inversely related to the levels of vitamin D₃ supplemented. The lack of significant effect of vitamin D₃ on feed intake may be due to the fact that lower levels of vitamin D₃ were supplemented compared with previous experiments of this series and with other studies.

The normal acid-base balance (blood pH, pCO₂, tCO₂, pCO₂, HCO₃⁻, BE and AG) of the steers was not altered by feeding a high Ca diet supplemented with 1500 mEq/d of anionic salts (magnesium sulfate and ammonium chloride) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU) for three days. It is possible that a mild metabolic acidosis was produced at this time in which the normal homeostatic mechanisms (renal excretion and respiratory rate) were able to buffer the excess of anions, such that the normal blood pH and the other parameters were maintained. No changes in blood pH have been observed in dry cows fed with acidified diets at 1200 mEq/d (Pehrson *et al.* 1999) or 2000 mEq/d (Oetzel *et al.* 1991). However, in the present study, when anionic salts were increased to 3000 mEq/d (fourth day) and vitamin D₃ supplementation levels maintained, the acid-base status was significantly altered. Blood pH, pCO₂, tCO₂, HCO₃⁻ and BE concentrations were significantly decreased. A reduction in these parameters is indicative of metabolic acidosis

induced by anionic salt supplementation, which could not be compensated by respiratory means.

It has been found that feeding acidified diets have resulted in higher plasma Ca concentrations in dairy cattle (Block 1984, 1994), in goats (Fredeen *et al.* 1988) and laying hens (Cohen *et al.* 1972). Total and ionized serum Ca concentrations were increased by anionic salt supplementation in this study by approximately 3 and 9 %, respectively. This response seems to be related to the induction of metabolic acidosis, which causes initially hypercalciuria (Tucker *et al.* 1992).

In this experiment, $1,25(\text{OH})_2\text{D}_3$ serum concentrations measured at the end of the supplemental period. At this point concentrations from treated animals (anion salts plus vitamin D_3) were higher than for control (anionic salts only) animals. Suggesting that vitamin D_3 supplementation induced a larger synthesis of $1,25(\text{OH})_2\text{D}_3$, which may be responsible for the observed increases in total and ionized serum Ca concentrations.

With respect to the objectives of this study, the most important effect of the anionic salt supplementation was that it helped to elicit the response in serum Ca to vitamin D_3 supplementation. For example, in the previous experiment (Chapter V), maximum total serum Ca concentrations for the 2.5 and 5 MIU treatments were 11.50 and 12.41 mg/dl, respectively, and in the present study they were 11.43 and 12.24 mg/dl for the 1.2 and 2.4 MIU treatments, respectively. That is, serum Ca concentrations were similar between experiments but now using only half of the amount of vitamin D_3 . On the other hand, ionized Ca concentrations in the previous experiment were 5.57 and 6.03 mg/dl for the 2.5 and 5 MIU treatment, respectively, and for this experiment they were 5.98 and 6.52 mg/dl for the 1.2 and 2.4 MIU treatments, respectively. In this case, the response was even higher, with ionized Ca levels exceeding the limits of 6.03 mg/dl from 5 MIU treatment in the previous experiment. The increase was approximately 0.5 mg/dl of ionized Ca over the 5 MIU treatment without anionic salts. This is a very interesting finding because the total serum Ca concentrations found in previous experiments for the 5 MIU treatment were in very good agreement with the range in which significant improvements in beef tenderization were obtained (Swanek *et al.* 1997, 1999; Montgomery *et al.* 1998, 2000; and Karges *et al.* 1999a,b, 2001). In the current trial, this same total Ca concentration was achieved with half of the supplemental vitamin D_3 (2.4 MIU/hd/d for ten days).

Furthermore, the increase in ionized Ca is critical as ionized or free Ca is the fraction of the total Ca in the extracellular fluid that can be transferred to other tissues. In particular, muscle tissue, where the high Ca levels can stimulate higher activity of the calpain proteolytic system and consequently enhance beef tenderness (Boehm *et al.* 1998; Geesink and Koohmaraie 1999; Montgomery *et al.* 2000).

These results suggest that by reducing the level of vitamin D₃ to 1.25 or 2.5 MIU/d in combination with the feeding of anionic salts at levels of 3000 mEq/d may be an alternative method of stimulating high serum Ca levels and to improve beef tenderness. However, before this can be stated with certainty, further research is required to determine the actual effect on the eating quality of beef and the potential for carcass residues of vitamin D₃.

Conclusions:

The low Ca diet appeared to be a feasible way to prime the Ca homeostatic mechanisms as feeding this diet resulted in lower total and ionized serum Ca concentrations, as well as plasma vitamin D₃ and 25(OH)D₃ concentrations. The acid-base status of the steers was not altered, however. Anionic salt supplementation effectively increased total and ionized Ca concentrations and helped to enhance the effect of vitamin D₃ supplementation, as serum Ca concentrations were similar or higher than the concentrations achieved with 5 MIU of vitamin D₃ per animal per day fed in previous experiments. This response of serum Ca was accomplished by the alteration of the normal acid-base status of the steers, which resulted in a mild metabolic acidosis that was not compensated for by respiratory means.

This response in serum Ca as a result of feeding anionic salts (3000 mEq/d) and vitamin D₃ (1.2 and 2.4 MIU/hd/d) is a possible alternative to feeding 5 MIU or greater amounts of vitamin D₃ to improve beef tenderness. Further research is required to define the benefits and/or limitations of such an approach.

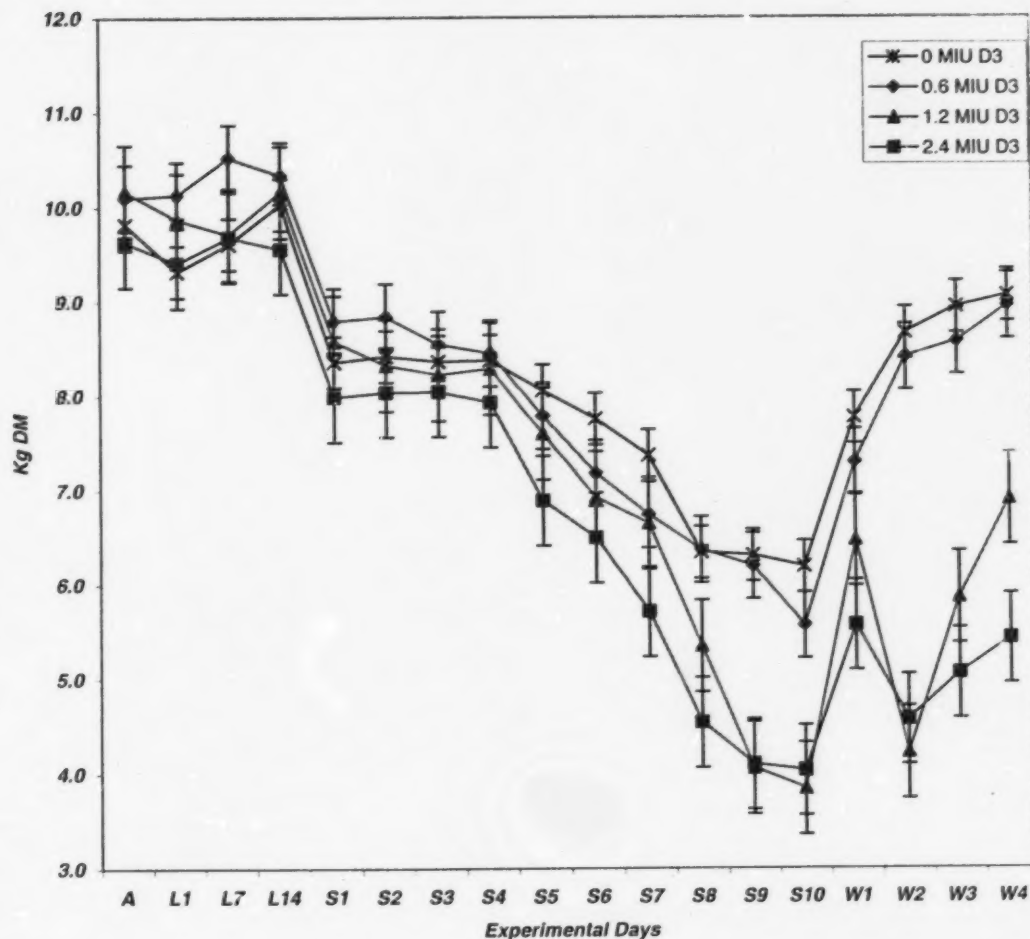


Figure 4.1. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/d) to a high Ca diet for ten days on feed intake (DMI) of finishing Hereford steers (448 ± 26 kg BW)(A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

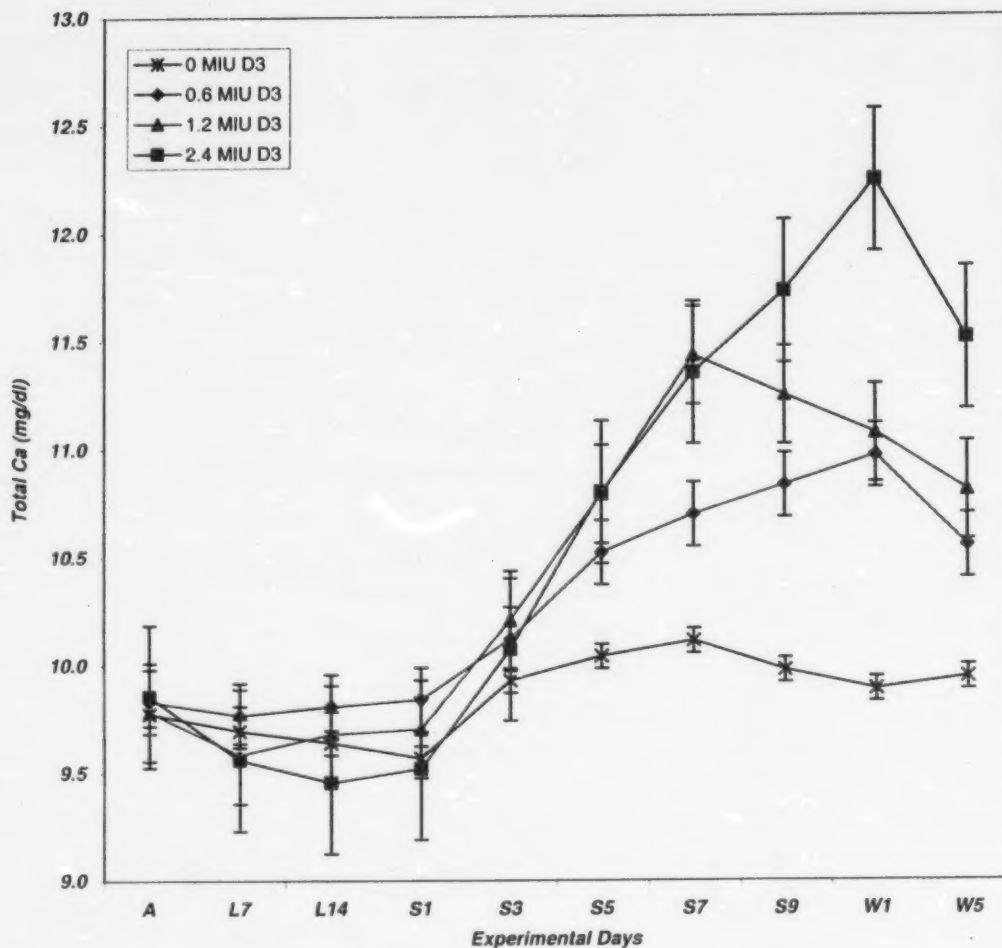


Figure 4.2. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/d) to a high Ca diet for ten days on total serum Ca concentrations of finishing Hereford steers (448 ± 26 kg BW)(A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

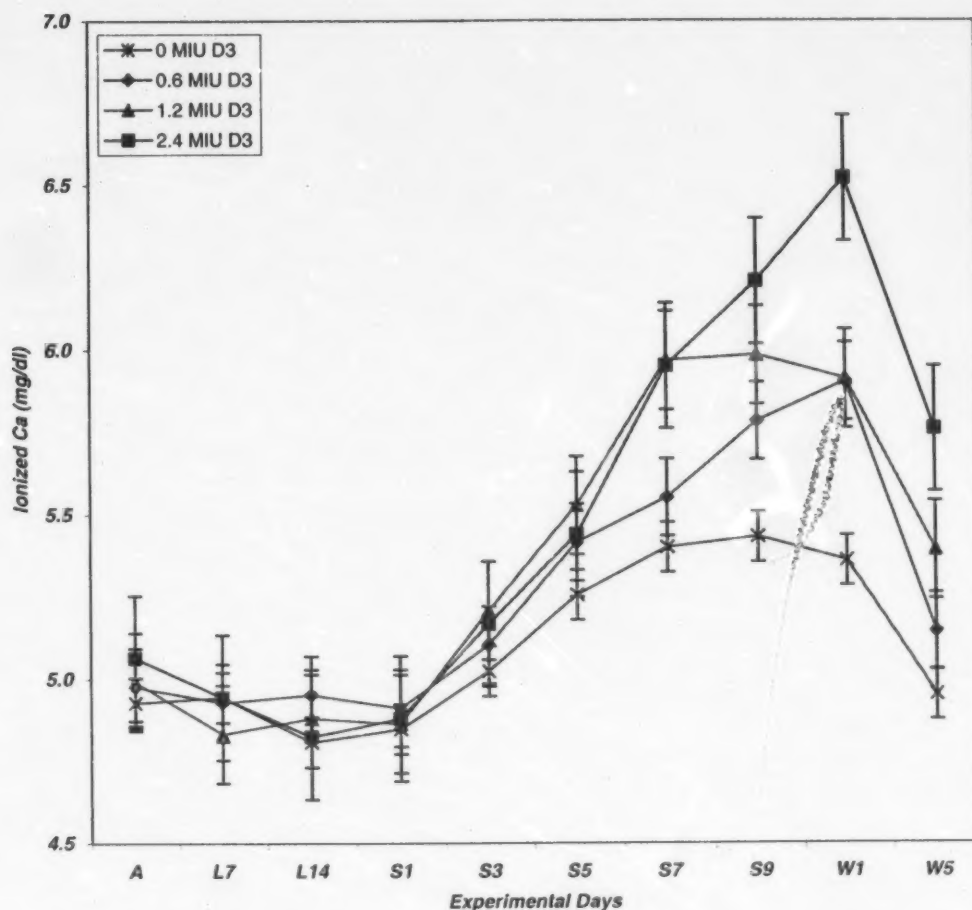


Figure 4.3. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/d) to a high Ca diet for ten days on ionized serum Ca concentrations of finishing Hereford steers (448 ± 26 kg BW)(A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

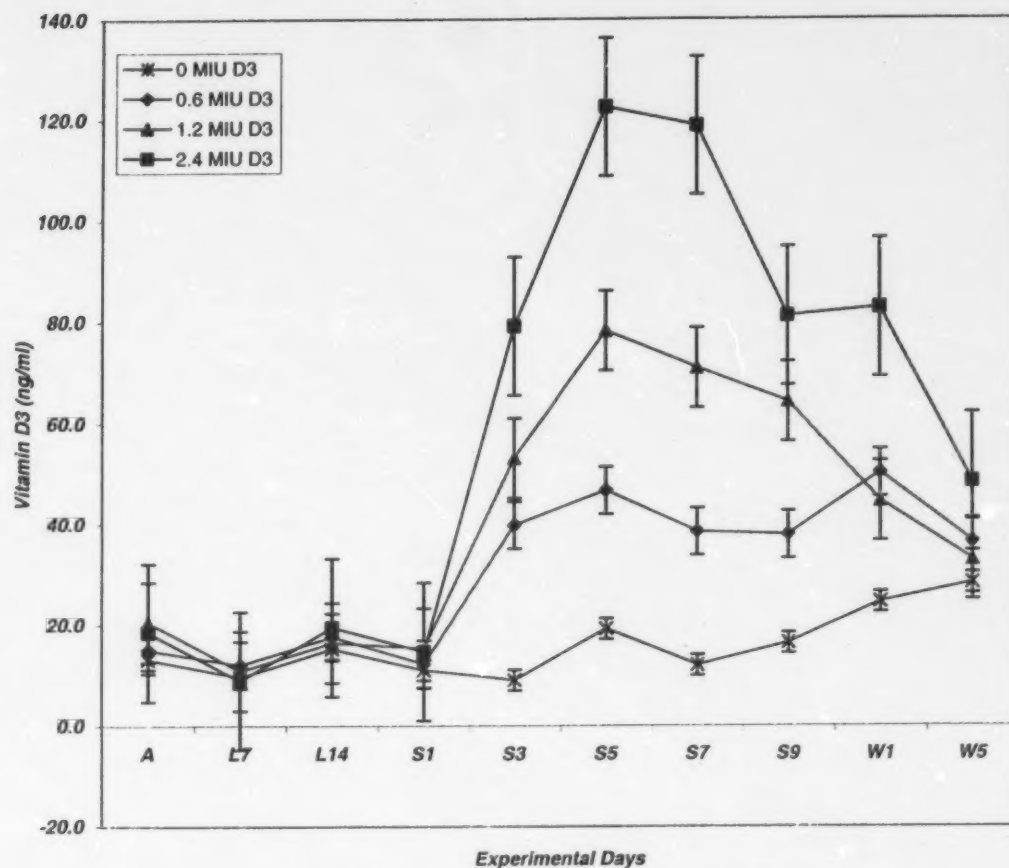


Figure 4.4. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/d) to a high Ca diet for ten days on plasma vitamin D₃ concentrations of finishing Hereford steers (448 ± 26 kg BW)(A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

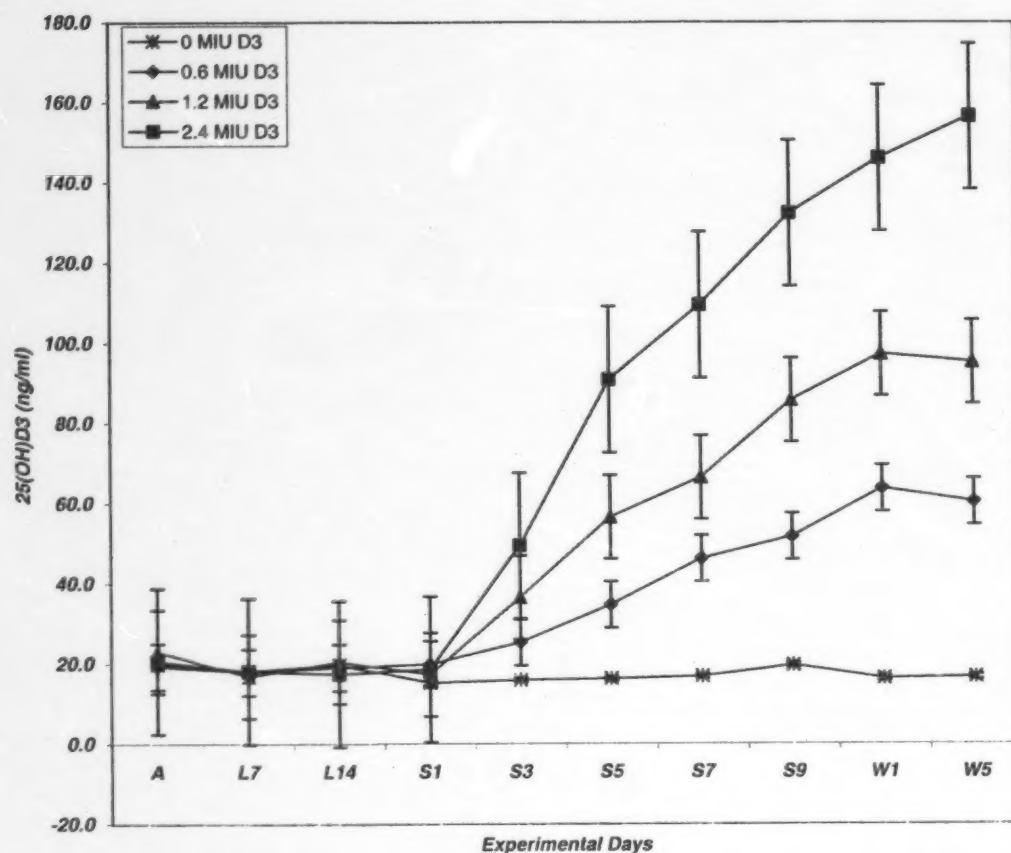


Figure 4.5. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/day) to a high Ca diet for ten days on plasma 25-hydroxyvitamin D₃ (25(OH)D₃) concentrations of finishing Hereford steers (448 ± 26 kg BW) (A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

Table 4.1. The effect of anionic salts and vitamin D₃ supplementation on circulating concentration of the calcitrophic hormones in finishing Hereford steers (448 ± 26 kg BW) at the first day of the withdrawal (W1) period.

Calcitrophic Hormones (pg/ml)								
Days	Vitamin D ₃ Level (MIU/hd/d)				SEM	Contrast ^z		
	0	0.6	1.2	2.4		A	B	C
Parathyroid Hormone (PTH):								
W1	21.22	12.92	4.14	4.32	2.20	<0.0001	0.0124	0.953
1,25-Dihydroxyvitamin D ₃ :								
W1	27.36	52.68	67.74	78.52	8.11	0.0007	0.2079	0.3616
Calcitonin:								
W1	304.85	300.55	291.63	313.80	16.02	0.8791	0.6990	0.3422

^z Contrasts:

A = Control (anion salts only) vs Vitamin D₃ treatments.

B = 0.6 vs 1.2 MIU D₃.

C = 1.2 vs 2.4 MIU D₃.

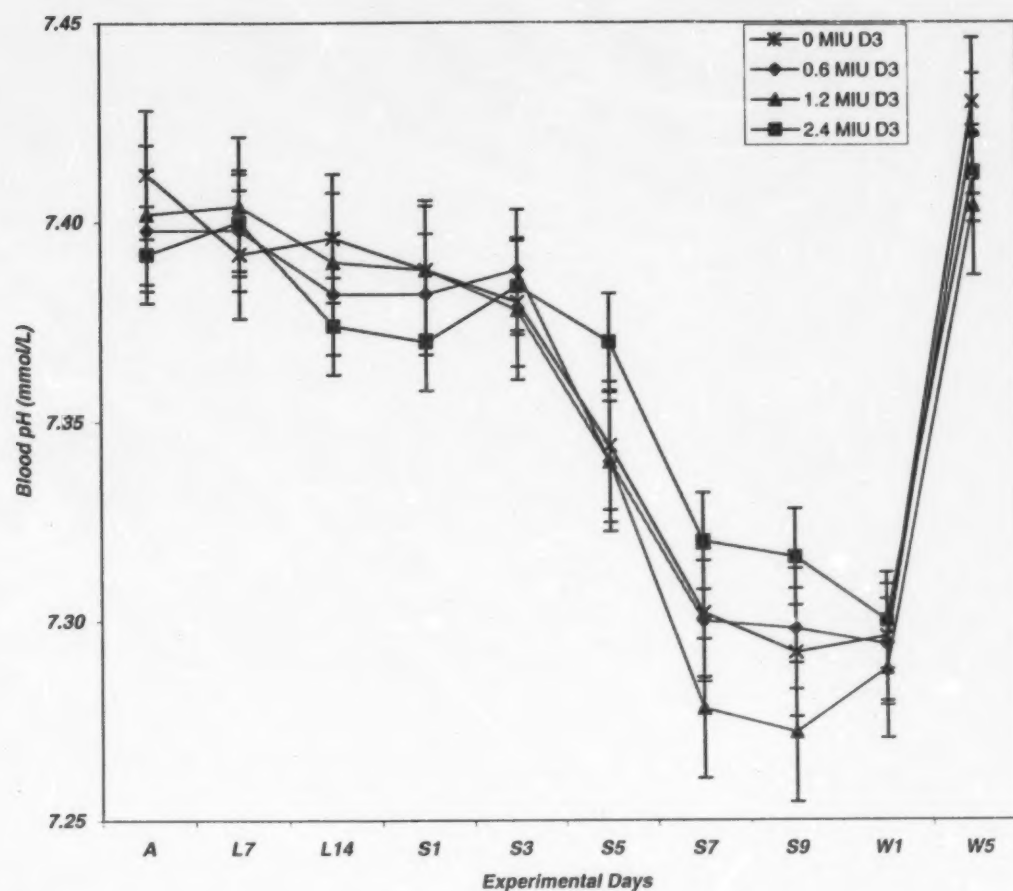


Figure 4.6. Effect of feeding a low Ca diet for fourteen days prior to supplementation of anionic salts (1500 and 3000 mEq/d) and vitamin D₃ (0, 0.6, 1.2 and 2.4 MIU/steer/day) to a high Ca diet for ten days on blood pH concentrations of finishing Hereford steers (448 ± 26 kg BW)(A=adaptation; L=low Ca; S=supplemental; and W=withdrawal periods).

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